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Expanding virus susceptibility spectrum of MDBK cells by expressing host receptors nectin 4 and TfR

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ARTICLE INFO

Keywords:

MDBK
TfR
Nectin 4
Host receptor
Susceptibility spectrum
Vaccine production

ABSTRACT

Cell-based vaccine manufacturing is a flexible and cost-effective approach for vaccine production which, however, requires cell adaptation to new vaccine strains. Generating one omnipotent or semi-omnipotent cell line feasible for the production of multiple viruses could help resolve this problem. We previously proposed virus Baltimore subtyping-based choice of receptors and a panel of minimally preferred receptors for the establishment of cells with a broad virus susceptibility spectrum. With the aim of establishing cells sensitive to viruses of livestock including bovine, ovine and canine, we selected TfR and Nectin 4 from the minimally preferred receptor panel, and successfully sensitized the starting cell line MDBK to CPV and CDV infection. Our study is a preliminary validation of our previously identified associations between host receptor usage and virus Baltimore subtyping. Evidence from more viruses of the same Baltimore subtyping and more starting cell lines need to be used to consolidate our results.

1. Introduction

Vaccination, providing immunity against one/several diseases for a time period or even the whole life span of an individual, is a prevalent approach for disease prevention including the current epidemic COVID-19 (Perdue et al., 2011). Since the 1930s, embryonated hen eggs were largely used to produce and manufacture human and veterinary vaccines (Goodpasture et al., 1931). However, egg-based manufacturing does not meet the increasing demand on vaccine production given its relatively low output. Further, the cost of producing egg-based manufacturing is generally high and the vaccinated individuals may generate potential allergic responses to egg components. Tissue culture approaches for vaccine production have been known since the early 1900s, when viruses such as vaccinia and mumps were shown to grow rapidly in the presence of fixed tissue explants (Perdue et al., 2011). This approach was pioneered by Enders, Wellers and Robbins for which they won the Nobel prize in 1954 (Salk et al., 1954). Accumulating evidence suggests that tissue culture technique is cost effective and flexible that allows for the production of multiple viral vaccines in the same production platforms (Perdue et al., 2011; Montomoli et al., 2012; Audsley

and Tannock, 2008). However, cell-based vaccines require adaptation of the vaccine strains to the new cells which sometimes proves to be difficult with variable outcomes (Genzel, 2015). Animal cells such as African green monkey kidney (Vero) cells, Madin Darby canine kidney (MDCK) cells, and chicken embryo fibroblasts (CEFs) have been used for viral vaccine production for years (Genzel, 2015). Among them, Vero cells have been used to produce vaccines against several diseases including Japanese encephalitis, dengue fever, West Nile encephalitis, Ross River fever, chikungunya fever, severe acute respiratory syndrome (SARS), smallpox, and influenza (Barrett et al., 2009). Given the keep emerging epidemic diseases caused by novel pathogens such as SARS-CoV-2, there is a demand on generating omnipotent or semi-omnipotent cell lines for the production of vaccines against a wide spectrum of viruses. Among the many factors known to affect cells' susceptibility to virus infection, adapting host receptors to enable successful virus infection was considered to be the key (Dai and Zhang, 2020).

We previously identified a panel of minimum preferred panel, i.e., TfR (transferrin receptor), Nectin 4 (nectin cell adhesion molecule 4), LDLR (low-density lipoprotein receptor), integrin, for adapting cells

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toward (semi-)omnipotent, and associated host receptor usage with virus Baltimore subtyping (Dai and Zhang, 2020). In this study, we are interested to experimentally validate these conceptual understandings through establishing a cell line feasible for the production of vaccines against diseases prevalent among livestock including bovine, ovine and canine including canine parvovirus (CPV), canine distemper virus (CDV), infectious bovine rhinotracheitis virus (IBRV), bovine diarrhea virus (BVDV) and bovine rotavirus (BRV). We chose Madin-Darby Bovine Kidney (MDBK) cells as the starting cell line which could be naturally infected by IBRV, BVDV and BRV given its natural expression of LDLR and integrin, and chose to express TfR and Nectin 4 on cell surface which are supposed to sensitize cells to a large proportion of +ssDNA (Baltimore subtype II) and -ssRNA (Baltimore subtype V) viruses, respectively. Our results showed that we had functionally expressed TfR and Nectin 4 on MDBK cells' surface that broadened their susceptibility spectrum to + ssDNA and -ssRNA viruses as represented by CPV and CDV. This study and our previously proposed associations between host receptor usage and virus Baltimore subtyping show our preliminary attempts towards the establishment of omnipotent cell lines for vaccine production that could be of great use for handling emergent scenarios such as the recent COVID-19 outbreak.

2. Materials and methods

2.1. Chemicals and antibodies

Fetal bovine serum (FBS) was purchased from Biological Industries Co., Ltd. (Israel); Trypsin, opti-MEM and Penicillin-streptomycin were purchased from Thermo Fisher Scientific (China) Co., Ltd. (United States); Dulbecco's modified Eagle's medium (DMEM) was purchased from HyClone Co., Ltd. (United States); Endonuclease was purchased from Thermo Fisher Scientific (China) Co., Ltd. (United States); Transfection reagent Lipofectamine 3000 and Lipofectamine 2000 were obtained from Thermo Fisher Scientific (China) Co., Ltd. (United States). Rabbit anti-FLAG tag antibody (HRP-66,003), murine anti-HIS tag antibody (HRP-66,005), HRP-labeled goat anti-rabbit IgG antibody (10285-1-AP), HRP-labeled goat anti-murine IgG antibody (10283-1-AP), FITC-labeled goat anti-rabbit IgG antibody (SA00003-2), FITC-labeled goat anti-murine IgG antibody (SA00003-1) were purchased from Proteintech Group Co., Ltd. (China). FITC-labeled murine anti-CDV antibody (CJ-F-CDV-MAB-1 mL or 10 mL) and FITC-labeled murine anti-CPV antibody (CJ-F-CPV-MAB-1 mL or 10 mL) were purchased from VMRD, Inc. (United States). TRIzol™ reagent and reverse transcriptase were purchased from Takara Biomedical Technology (Beijing) Co., Ltd. (Japan). RIPA lysis buffer and BCA Protein Assay Kit were purchased from Beyotime Biotechnology Co., Ltd. (China).

2.2. Cells and viruses

MDBK, Madin-Darby Canine Kidney (MDCK) cells and Crandell Feline Kidney (CFK) cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. CDV (NCBI:txid

11,232) and CPV (NCBI:txid 10,788) were purchased from the China Veterinary Culture Collection Center.

2.3. Cell culture and virus reproduction

MDBK, MDCK and CFK cells were cultivated in DMEM supplemented with 10 % FBS and 1% penicillin-streptomycin, and grown at 37 °C and 5%(v/v) CO₂ in a humidified incubator. CPV was inoculated to cells before cell adhesion and cultured together using low FBS medium (DMEM containing 2% FBS). Cytopathic effect (CPE) was detected 5 days later after CPV synchronous inoculation. After cell adhesion, cells were cultured using low FBS medium, followed by CDV inoculation. CPE was monitored 3 days later after CDV asynchronous inoculation.

2.4. Plasmid construction

The canine TfR mRNA sequence (GenBank No: NM_001003111.1) was retrieved from Genbank. The Kozak sequence (5'-GCCACCAUGG-3'), IgK single peptide sequence, and restriction site *Bam*HI were added to the 5' end of the start codon ATG, and 6 × HIS Tag, stop codon TGA and restriction site *Eco*RI were added to the 3' end of the coding sequence. Codon optimization and protein-coding sequence synthesis were performed by Jiangsu Yixin Biotechnology Co., Ltd.. The synthesized sequence was ligated into the vector pLVX to construct the pLVX-DogTfR-His plasmid.

The canine Nectin 4 mRNA sequence (GenBank No: NM_001313853.1) was retrieved from Genbank. The Kozak sequence, IgK single peptide sequence, and restriction site *Eco*RI were added to the 5' end of the start codon ATG, and a Flag tag, stop codon TGA and restriction site *Xba*I were added to the 3' end of the coding sequence. Codon optimization and protein-coding sequence synthesis were performed by Jiangsu Yixin Biotechnology Co., Ltd.. The synthesized sequence was ligated into the vector pcDNA3.1 (+) to construct the pcDNA3.1 (+)-DogN4-Flag plasmid.

2.5. Double digestion

0.5 μL *Bam*HI, 0.5 μL *Eco*RI, 1 μL pLVX-DogTfR-His and 8 μL ddH₂O were mixed in a 10 μL centrifuge tube and put in a 37 °C water bath for 30 min. 0.5 μL *Eco*RI, 0.5 μL *Xba*I μL, 1 μL pcDNA3.1 (+)-DogN4-Flag and 8 μL ddH₂O were mixed in a 10 μL centrifuge tube and put in a 37 °C water bath for 30 min.

2.6. Cell transfection

The efficiencies of three transfection approaches, i.e., calcium phosphate, liposome, and electroporation, were compared for optimal MDBK cell transfection. MDBK transfected with pLVX-DogTfR-His and with pcDNA3.1 (+)-DogN4-Flag were named as MDBK-TfR and MDBK-N4, respectively.

Calcium phosphate transfection: Refresh the medium when MDBK cells grow to 80 % confluence in a 6-well plate. Mix 9 μL of 2 m/L CaCl₂, 5 μg of DNA, and 86 μL ddH₂O as Solution A. Take 100 μL 2 × HBS as Solution B. Add solution A in a dropwise fashion to Solution B, mix them in a 6-well plate and place the it in an incubator overnight. After 24 h, discard the supernatant, wash the precipitates twice with PBS, replace it with fresh medium and culture it for 24 h. Cells were collected for detection.

Liposomal transfection: Refresh the medium with serum-free medium when MDBK cells grow to 80 % confluence in a 6-well plate. Add 3.75 μL lipofectamine 3000 or 8 μL lipofectamine 2000 to 100 μL opti-MEM to prepare Solution A. Add 2.5 μg plasmid to 100 μL opti-MEM to prepare Solution B. Mix Solution A and Solution B and incubate it at the room temperature for 15 min. Centrifuge cells and aspirate the supernatant. Place the precipitates in the mixture of Solution A and Solution B, and incubate it for 6–8 h followed by cultivation using

Table 1
Primers of genes used in this study.

Primers name	Primers sequence
RT-PCR-dog- <i>Nectin4</i> -F	ACGCGATTCTCAGGTGACAGT
RT-PCR-dog- <i>Nectin4</i> -R	AACAGCAGTGGCGCAATAAC
RT-PCR-dog- <i>TfR</i> -F	GCCACCATGATGGATCAA
RT-PCR-dog- <i>TfR</i> -R	TCATTAGTGATGGTGATGGT
RT-PCR-cattle- <i>GAPDH</i> -F	CCTGCCCGTTCGACAGATAG
RT-PCR-cattle- <i>GAPDH</i> -R	ATGGCGACGATGTCCACTTT
PCR-CDV-F	TGGCTAGCCTTCTTAAGAGC
PCR-CDV-R	TTAATTGAGTAGCTCTGTTCATTA
PCR-CPV-F	ATGAGTGATGGAGCAGTCA
PCR-CPV-R	TTAATATAATTTTCTAGGTGCTAGT

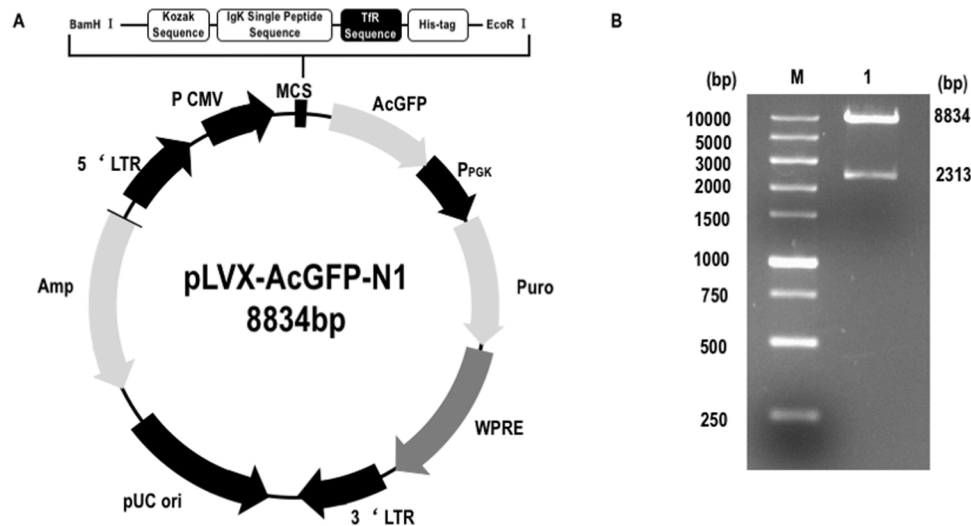


Fig. 1. Plasmid Design of pLVX-DogTfR-His and Agarose Gel after Double Endonuclease (*Bam*HI and *Eco*RI) Cutting. (A) Plasmid design of pLVX-DogTfR-His. (B) Agarose gel after double endonuclease (*Bam*HI and *Eco*RI) cutting.

normal medium for 48 h. Cells were collected for detection.

Electroporation transfection: Trypsinize and resuspend cells using pre-chilled PBS when cells reach 100 % confluence. Resuspend 2×10^6 cells in 200 μ L PBS. Add 20 μ g plasmid and 10 μ g salmon sperm DNA in a pre-chilled 2 mm electric shock cup for 1 min ice-bath. Set the cell electroporator at 350 V and treat cells 500 μ s for 3 shocks at an interval of 1 min. Cells were washed with DMEM containing 10 % FBS and transferred to a 6-well plate with a final volume of 2 mL/well. Cells were collected for detection after 24 h.

2.7. PCR

Take 2 μ L template, 0.5 μ L upstream and 0.5 μ L downstream primers (Table 1), 2 μ L ddH₂O, 5 μ L PrimerStarMax mix enzyme (Takara), and mix them in a 200 μ L centrifuge tube. The PCR program was the same for both TfR and Nectin 4, and was set as 94 °C for 2 min, 94 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, for 35 cycles. The primers were self-designed using Primer5 and synthesized from GeneWiz (Suzhou, China). PCR was used to validate the correctness of plasmid construction.

2.8. qPCR

Total RNA was collected from the tissues or cells using TRIzol™ reagent and reverse transcribed to cDNA by reverse transcriptase following the manufacturer's protocol (Takara). Mix 2 μ L template, 0.5 μ L upstream and 0.5 μ L downstream primers (Table 1), 2 μ L ddH₂O, 4.8 μ L Takara mix enzyme and 0.2 μ L ROX (6-carboxy-X-rhodamine), in a 200 μ L qPCR centrifuge tube. The qPCR program was the same for both TfR and Nectin 4, and was set as 95 °C for 10 min, 95 °C for 10 s, 60 °C for 60 s, 72 °C for 20 s, for 40 cycles. Target gene expression was evaluated using the $2^{-\Delta\Delta C_t}$ relative quantification method and normalized by GAPDH expression. Each reaction was repeated at least three times independently. The primers were self-designed using Primer5 and synthesized from GeneWiz (Suzhou, China). The qPCR assay was used to quantify gene expression level.

2.9. Western blot

Cells were washed twice with pre-chilled PBS and lysed on ice for 1 min in RIPA lysis buffer supplemented with protease and phosphatase inhibitors. Cells were centrifuged at 12,000 g for 5 min followed by supernatant collection. The protein concentration was quantified by the BCA Protein Assay Kit. Proteins from each lane (50 μ g) were separated

by SDS-PAGE and transferred to a PVDF membrane. Non-specific binding sites were blocked for 1 h at the room temperature using 0.05 % (v/v) Tween 20 and 5% (w/v) skim milk containing 1 x TBS. After washing, the membrane was incubated with the specific primary antibody for at least 12 h at 4 °C and with a suitable horseradish peroxidase-conjugated secondary antibody for 1.5 h at the room temperature. The reaction protein bands were visualized using Tanon™ High-sig ECL Western Blotting Substrate and the expression levels of the reactive protein bands were quantified using Image J software.

2.10. Direct immunofluorescence

Direct immunofluorescence assay was used to assess the cells' infectivity to virus infection. The fluorescent signal was tagged on the primary antibody in direct immunofluorescence. Cells were cultured in 6-well plates. MDBK-N4 and MDBK cells were infected with CDV with an MOI (Multiplicity of Infection) of 0.1, and MDBK-TfR and MDBK were infected with CPV at an MOI of 0.1. When cells began to appear CPE, they were fixed with 4% formaldehyde for 10 min at 4 °C followed by pre-chilled PBS wash; cells were treated with 0.3 % Triton-X for 10 min and washed again using pre-chilled PBS. MDBK-N4, MDBK and MDCK cells were incubated with the CDV antibody, and MDBK-TfR and MDBK and CFK cells were incubated with the CPV antibody for 12 h. Fluorescence was detected using confocal laser scanning microscopy (Zeiss).

2.11. Indirect immunofluorescence assay

Indirect immunofluorescence assay was used to examine receptor expression. Both primary and secondary antibodies were needed in indirect immunofluorescence, where fluorescent signal was tagged on the secondary antibody. MDBK and CFK cells were grown in 6-well plates. After blocking with 1% bovine serum albumin (BSA) for 30 min, cells were incubated with different primary antibodies (Flag, His, 1:300) for 8 h, following treatment with corresponding fluorescent secondary antibodies for additional 1 h. Goat anti-rabbit/murine IgG (H + L) secondary antibody (1:500) was used. Cells were photographed by a laser-scanning confocal microscope (Zeiss).

2.12. Median tissue culture infective dose measurement

Viruses were inoculated into cells for 5 days till CPE generation. The supernatant from the virus solution was taken to calculate the Median Tissue Culture Infective Dose (TCID₅₀).

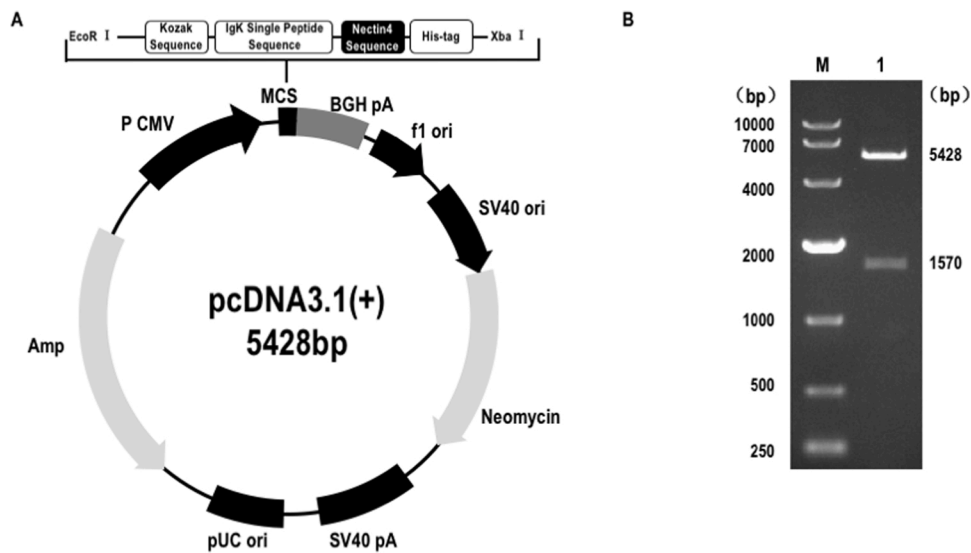


Fig. 2. Plasmid Design of pcDNA3.1 (+)-DogN4-Flag and Agarose Gel Imaging after Double Endonuclease (*EcoRI* and *XbaI*) Cutting. (A) Plasmid design of pcDNA3.1 (+)-DogN4-Flag. (B) Agarose gel imaging after double endonuclease (*EcoRI* and *XbaI*) cutting.

2.13. Flow cytometry

Take 2 flow detection tubes for each group, label them as blank-tube and testing-tube. Add 10^6 cells/100 μ L to each tube respectively. Add cell washing solution to the blank tube, and the primary antibody of FLAG tag and HIS tag to testing-tube. After incubating them at 4 $^{\circ}$ C for 30 min, centrifuge at 1200 r/min for 10 min, and wash them twice. Add fluorescently labeled secondary antibody to each tube and mix them. After incubating at 4 $^{\circ}$ C for another 30 min, centrifuge at 1200 r/min for 10 min, and wash twice, too. Then, remove the supernatant and add 0.5 mL fixative to each tube and mix well. When testing on the BD Accuri C6 flow cytometry, test the blank tube firstly to adjust the voltage to the negative zone, and then test the testing-tube. The results were analyzed by FlowJo V10.

2.14. Passaging assay

MDBK-Tfr and MDBK-N4 cells were seeded in the 6-well plate at a density of 3×10^5 /well (marked as Passage 1). The cells were digested and passaged at a ratio of 1:3 each 2 days, marked as Passage 2, 3, 4, 5. The cells of Passage 1, 3, 5 were extracted proteins for WB detection.

2.15. Statistical analysis

The percentage of transfection from immunofluorescence imaging was quantified by Image J. Virus infectivity was assessed using TCID50 and qPCR cycle threshold. Statistical analyses were conducted by SPSS software 25.0. The data of different groups were analyzed using two-way student T test, with $p < 0.05$ being considered with statistical significance.

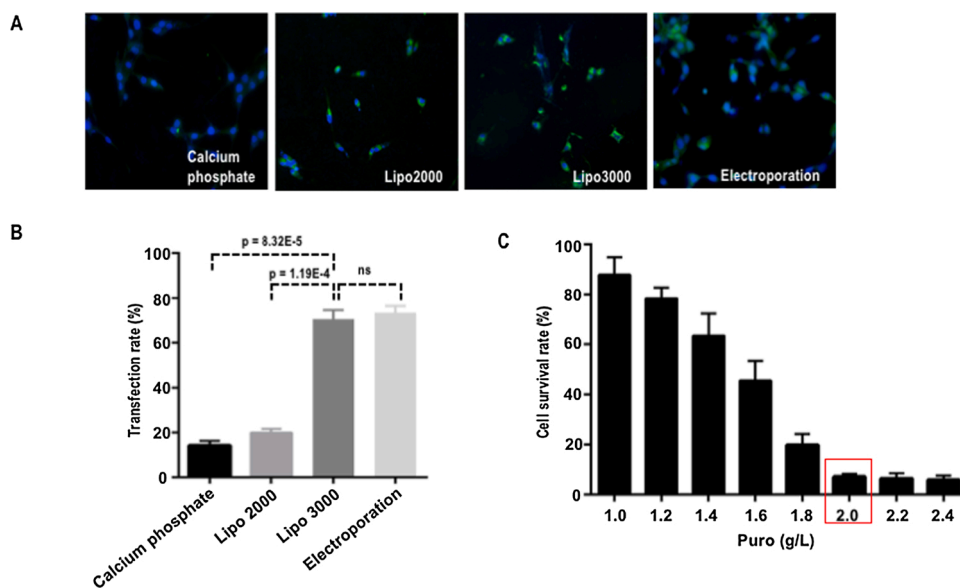


Fig. 3. Comparisons on Transfection Efficiency among Different Transfection Approaches in MDBK. (A) Immunofluorescence imaging, and (B) transfection rates of calcium phosphate, lipofectamine 2000/3000, and electroporation mediated plasmid transfection in MDBK cells. (C) Cell survival rates after 7 days cultivation under different puromycin antibiotic concentrations.

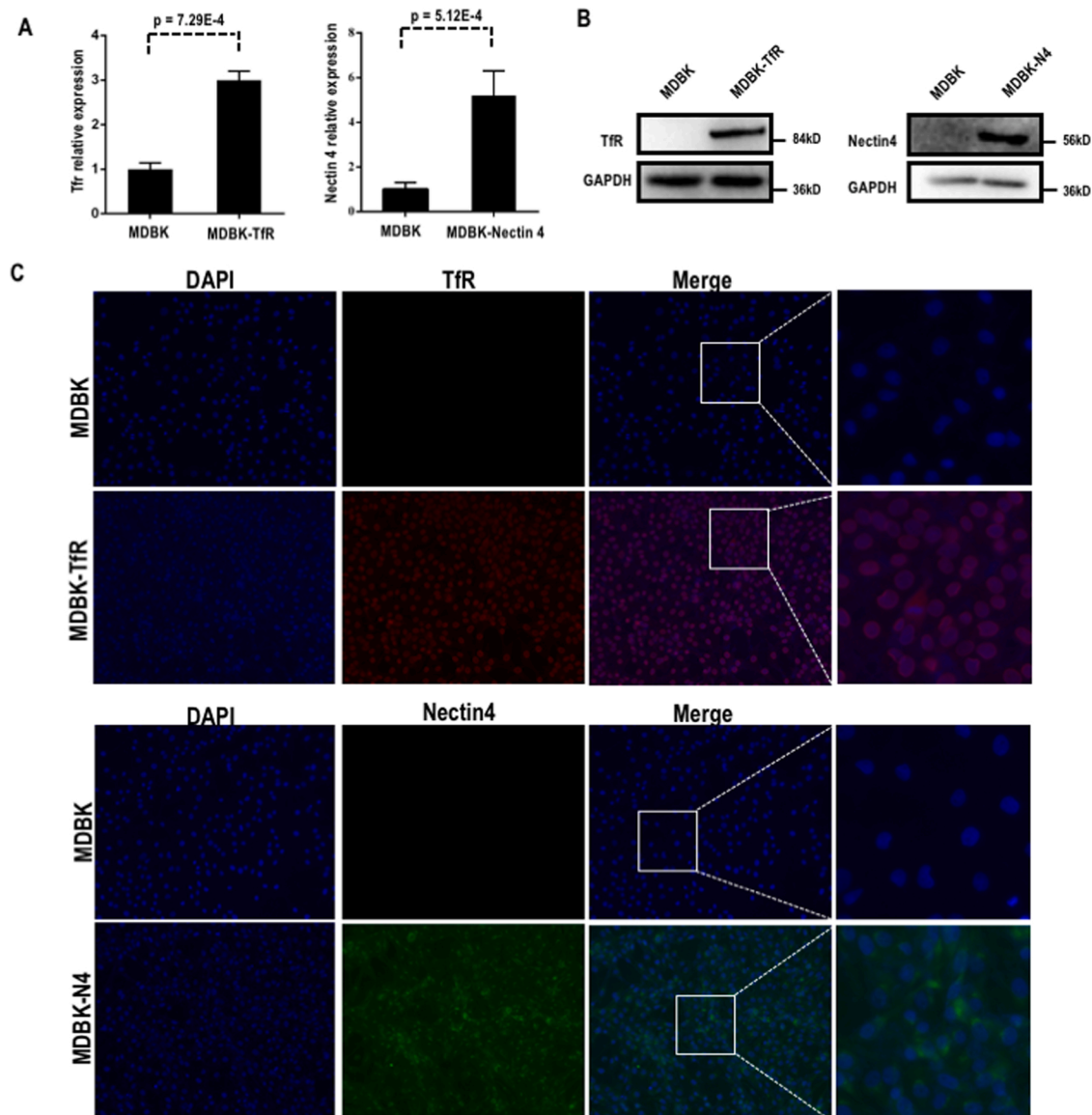


Fig. 4. Receptor Expression and Localization after Plasmid Transfection. (A) qPCR, (B) western blot, and (C) immunofluorescence imaging showing receptor expression and localization after plasmid transfection.

3. Results

3.1. Plasmid construction and identification

The plasmid pLVX-DogTfR-His (Fig. 1A) was cut by restriction enzymes *Bam*HI and *Eco*RI, with the 8834 bp and 2313 bp bands being the pLVX-DogTfR-His plasmid backbone and the inserted fragment including the TfR sequence, respectively (Fig. 1B).

The plasmid pcDNA3.1 (+)-DogN4-Flag (Fig. 2A) was cut by restriction enzymes *Eco*RI and *Xba*I, with the 5428 bp and 1570 bp bands being the pcDNA3.1 (+)-DogN4-FLAG plasmid backbone and the inserted fragment including the Nectin 4 sequence, respectively (Fig. 2B).

3.2. Comparison on the transfection efficiency of different transfection methods

Calcium phosphate transfection, liposome transfection (lipofectamine 2000/3000), and electroporation transfection approaches, were used to transiently transfect the plasmids into MDBK cells.

Lipofectamine 3000 and electroporation were shown to have a comparable transfection efficiency that was considerably higher than that of the calcium phosphate and lipofectamin 2000 approaches (Fig. 3A, B). Lipofectamine 3000 transfection was used in the following experiments.

Stable cells were selected under puromycin pressure. After 7 days culture in medium containing various concentrations of puromycin, cell survival rate approached approximately 8% at a puromycin concentration of 2 g/L or above (Fig. 3C), which was used as the selection concentration.

3.3. Expression and localization of expressed receptors

As compared with MDBK cells that showed no or weak TfR or Nectin 4 expression, MDBK-TfR and MDBK-N4 cells showed considerably stronger TfR and Nectin 4 expression, respectively (Fig. 4A, B). Immunofluorescence imaging showed that both TfR and Nectin 4 were expressed on the surface of MDBK-TfR and MDBK-N4, respectively (Fig. 4C).

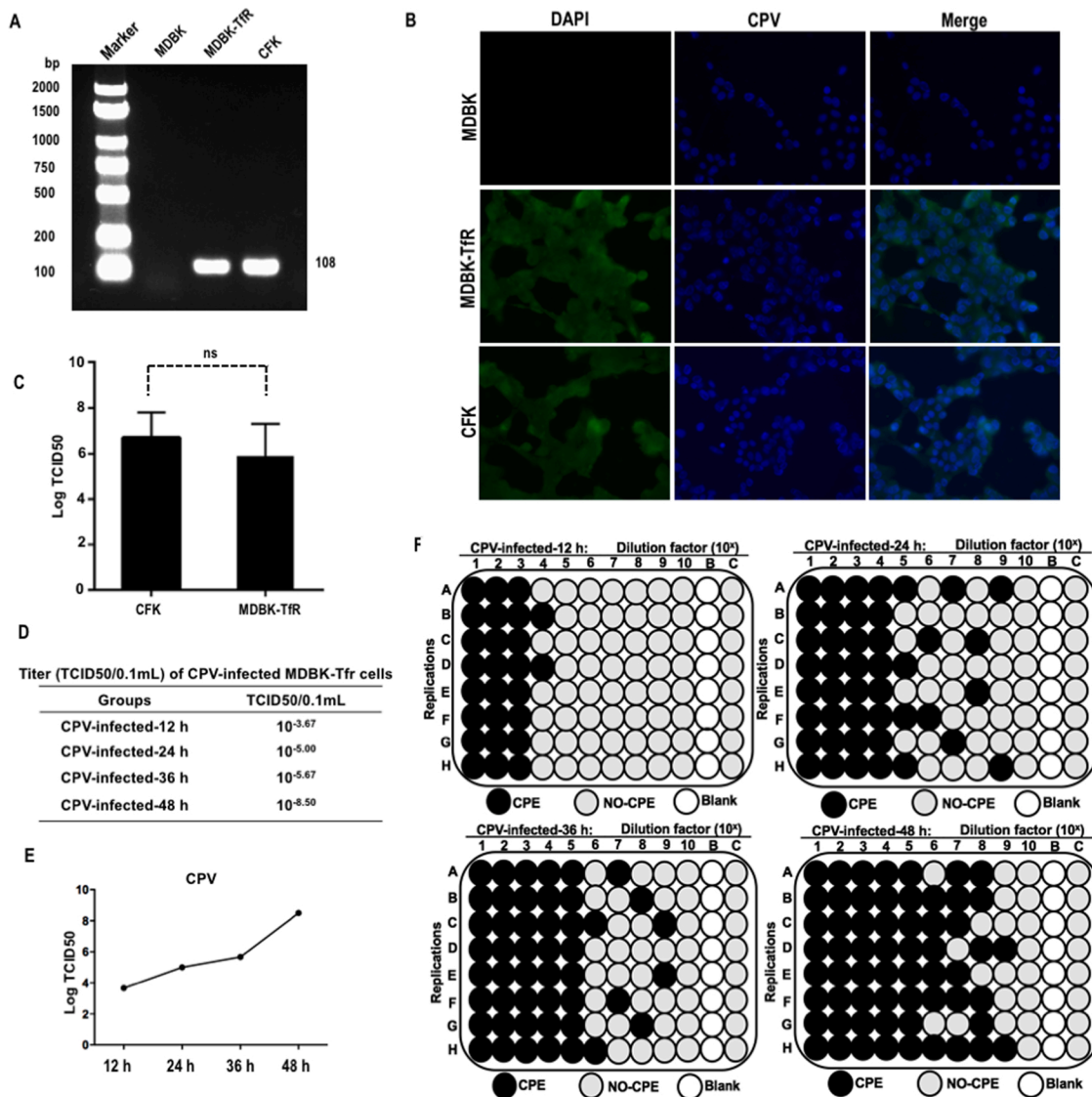


Fig. 5. Virus Infection Detection in MDBK Cells Constructed with TfR. (A) Agarose gel, (B) immunofluorescence imaging, (C) TCID50 and (D) qPCR circle threshold showing CPV infection on MDBK-TfR cells.

3.4. Functionality of expressed receptors

As compared with MDBK cells, MDBK-TfR cells showed a band of CPV nucleic acid with comparable intensity (Fig. 5A) and TCID50 (Fig. 5C) with CFK. TCID50 of CPV increased with time (Fig. 5D–5F). The FITC-labeled murine anti-CPV antibody was shown on the surface or inside MDBK-TfR and CFK cells but not MDBK cells (Fig. 5B).

As compared with MDBK cells, both MDBK-N4 and MDCK cells showed a band of CDV nucleic acid (Fig. 6A) and comparable TCID50 (Fig. 6C). TCID50 of CDV increased with time (Fig. 6D–6F). The FITC-labeled murine anti-CDV antibody was shown in the cytoplasm of MDBK-N4 and MDCK cells but not MDBK cells (Fig. 6B).

3.5. Clonability and stability of MDBK-TfR and MDBK-N4 cells

Through flow cytometry, we found that 97.5 % MDBK-TfR and 94.4 % MDBK-N4 cells expressed TfR and N4, respectively, whereas the control MDBK cells expressed 5.42 % TfR and 2.24 % N4 (Fig. 7A), suggesting the high clonability of both MDBK-TfR and MDBK-N4 cells.

The expression intensities of TfR and N4 as assessed using western blot were not reduced in MDBK-TfR and MDBK-N4 cells after 5 passages (Fig. 7B), suggesting high stability of both constructed cells.

4. Discussion

In this study, we showed that by expressing TfR and Nectin 4, respectively, on the surface of MDBK cells, we could successfully sensitize MDBK cells to CPV and CDV infection. The choice of receptors was based on the minimum preferred receptor panel presented in (Dai and Zhang, 2020), where we classified receptors into different groups according to virus Baltimore subtyping. CPV and CDV are + ssDNA and –ssRNA viruses, which belong to the Baltimore subtypes II and V respectively. According to our systematic analysis covering both human and animal viruses, we found that TfR and Nectin 4 were the most prevalent host receptors used by viruses of Baltimore subtypes II and V respectively. Thereby, TfR and Nectin 4 were selected to represent these two classes of viruses and included in the minimum preferred receptor panel for the establishment of potential omnipotent cell lines with broad virus susceptibility spectrum (Dai and Zhang, 2020). This study selected host receptors based on virus Baltimore subtyping according to our previously identified associations between host receptor usage and virus characteristics (Dai and Zhang, 2020) and, thus, is an *in vitro* validation of our previous concepts. The successful recipes here could be transferred to other studies attempting to expand the virus susceptibility spectrum of cells for the purpose of, e.g., vaccine production.

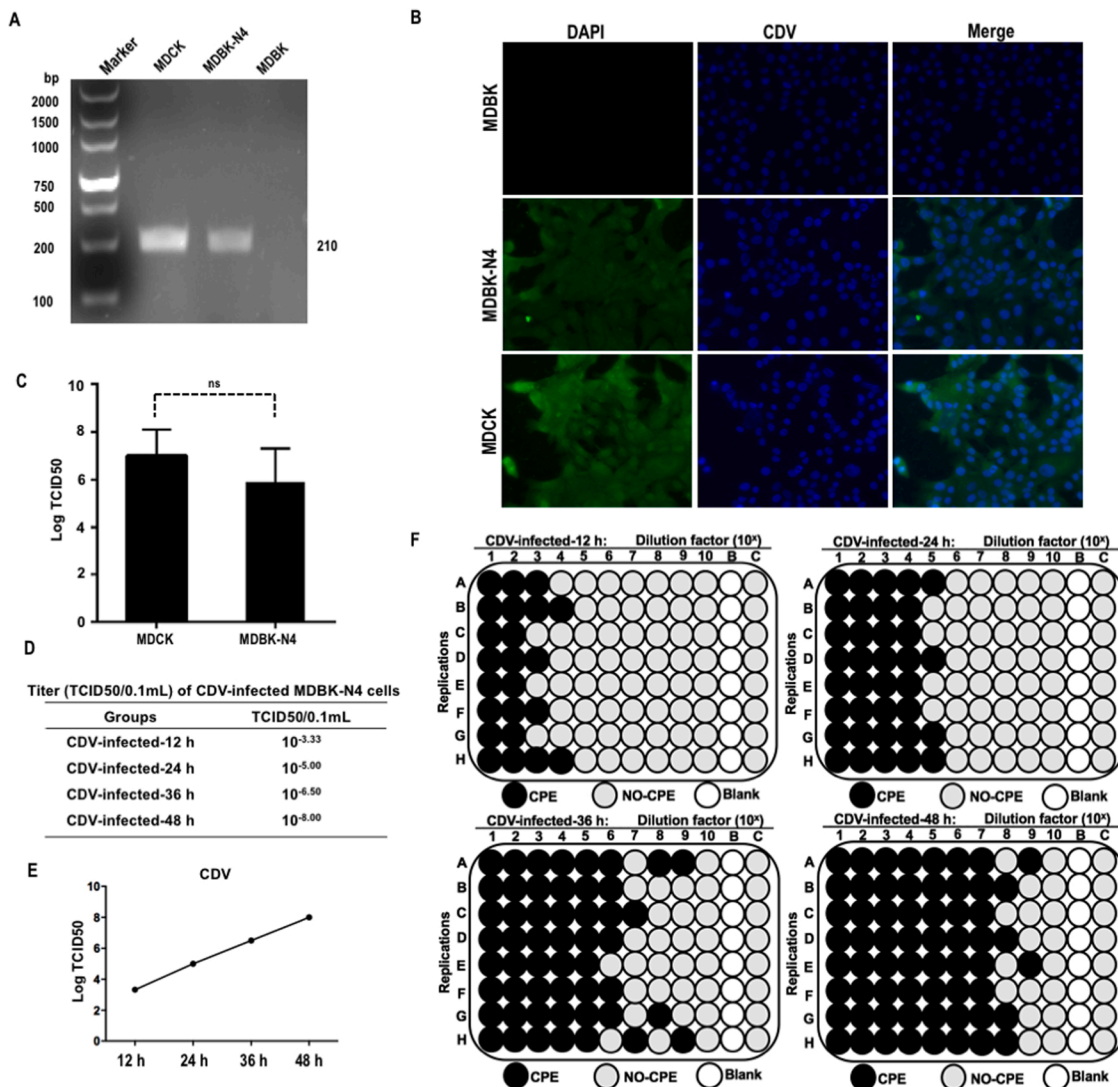


Fig. 6. Virus Infection Detection in MDBK Cells Constructed with Nectin 4. (A) Agarose gel, (B) immunofluorescence imaging, (C) TCID50 and (D) qPCR circle threshold showing CDV infection on MDBK-N4 cells.

Though this study explored the feasibility of using TfR and Nectin 4 in mediating the infection of + ssDNA and -ssRNA viruses as represented by CPV and CDV, respectively, we did not co-express them in one cell system, and thus did not investigate the potential interference between TfR and Nectin 4 as well as between externally expressed receptors and already existing receptors on cell surface. Also, more types of + ssDNA and -ssRNA viruses need to be tested in MDBK-TfR and MDBK-N4 cells, and more starting cell lines need to be used to consolidate our conclusions on the intrinsic connections between virus Baltimore subtyping and host receptors. These constitute our future efforts.

We used animal instead of human viruses in this study to overcome limitations from our available facilities and meet the regulatory restrictions on experiments involving viruses. Lastly, we would like to emphasize the importance of codon optimization and the choice of transfection approach in cell surface modification which are likely to be cell type specific and impinge on the ought-to-be output if not optimized.

5. Conclusion

We successfully expressed TfR and Nectin 4, respectively, on the surface of MDBK cells and sensitized them to CPV and CDV infection. The choice of TfR and Nectin 4 was based on CPV and CDV Baltimore

subtyping and our previous conceptual knowledge on the associations between virus features and host receptor usage. This study provided preliminary validations on the feasibility of our previously proposed panel of minimally preferred receptors towards the establishment of potential (semi-)omnipotent cells for producing vaccines against a wide spectrum of viruses, and shed light on novel vaccine development strategies for coping with urgent epidemic viral disease outbreaks such as the recent COVID-19. However, evidence from more viruses and cells need to be provided to consolidate the identified associations between virus Baltimore subtyping and host receptors, which still require intensive efforts.

Funding

This study was funded by the National Natural Science Foundation of China (Grant No. 81972789), the National Science and Technology Major Project (Grant No. 2018ZX10302205-004-002), Technology Development Funding of Wuxi (Grant No. WX18IVJN017), Fundamental Research Funds for the Central Universities (Grant No. JUSRP22011). These funding sources have no role in the writing of the manuscript or the decision to submit it for publication.

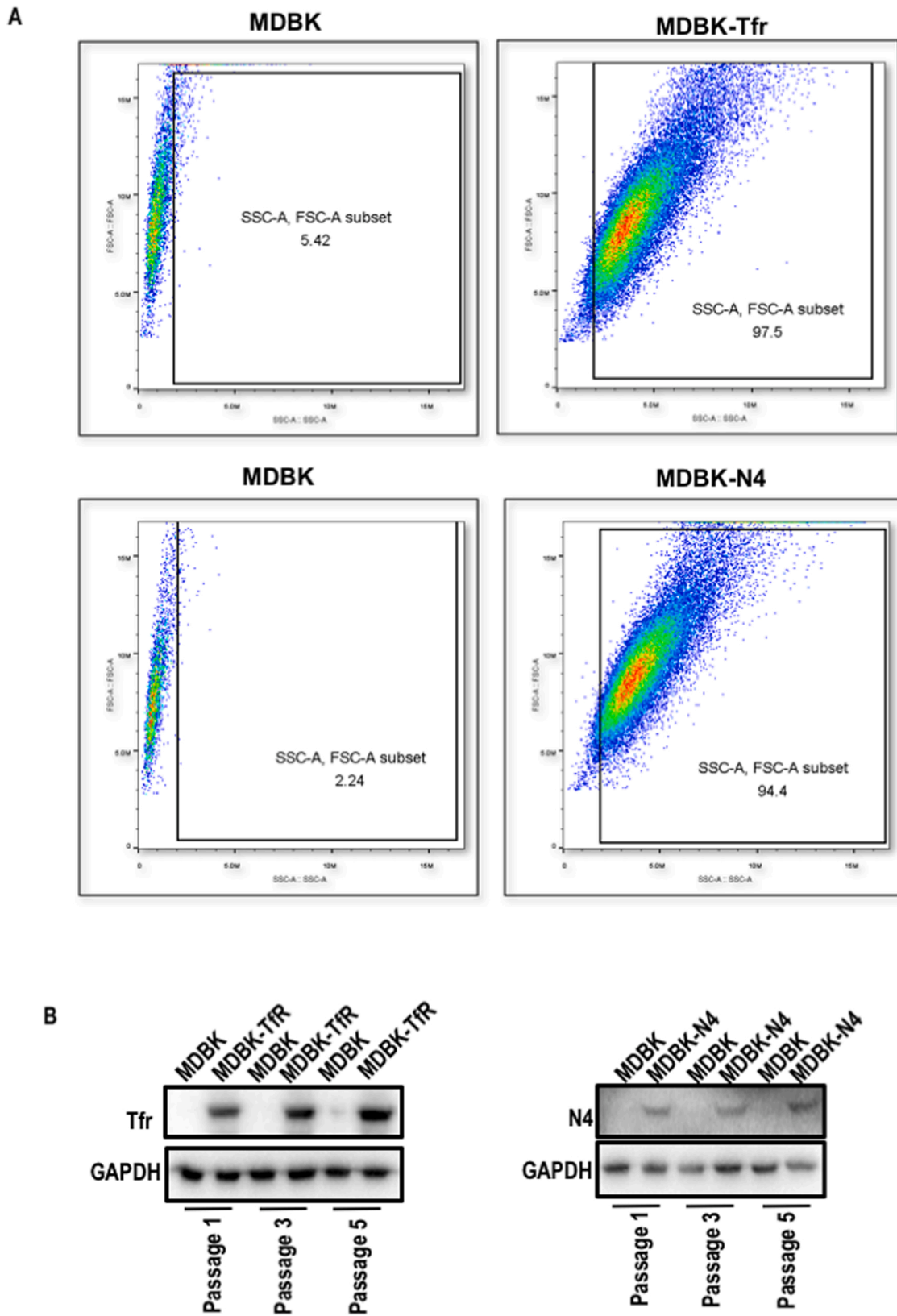


Fig. 7. Clonability and stability of MDBK-Tfr and MDBK-N4. (A) Flow cytometry detecting Tfr and Nectin 4 in MDBK-Tfr and MDBK-N4 cells. (B) Tfr and Nectin 4 expression during cell passaging.

Ethics approval and consent to participate

Not applicable.

Consent for publication

The authors are consent for its publication.

Availability of data and materials

Not applicable.

Authors' contributions

XFD selected the topic, initiated the project, conceptualized the ideas, and drafted the manuscript. PYH, XHZ and SMY conducted the experiments, and prepared the figures and tables. All authors have approved the content of the manuscript for publication.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

Not applicable.

References

- Audsley, J.M., Tannock, G.A., 2008. Cell-based influenza vaccines: progress to date. *Drugs* 68, 1483–1491.
- Barrett, P.N., Mundt, W., Kistner, O., Howard, M.K., 2009. Vero cell platform in vaccine production: moving towards cell culture-based viral vaccines. *Expert Rev. Vaccines* 8, 607–618.
- Dai, Xiaofeng, Zhang, Xuanhao, Ostrikov, Kostya(Ken), Abrahamyan, L., 2020. Host receptors: the key to establishing cells with broad viral tropism for vaccine production. *Crit. Rev. Microbiol.* 46, 147–168.
- Genzel, Y., 2015. Designing cell lines for viral vaccine production: where do we stand? *Biotechnol. J.* 10, 728–740.
- Goodpasture, E.W., Woodruff, A.M., Buddingh, G.J., 1931. The cultivation of vaccine and other viruses in the chorioallantoic membrane of chick embryos. *Science* 74, 371–372.
- Montomoli, E., Khadang, B., Piccirella, S., Trombetta, C., Mennitto, E., Manini, I., Stanzani, V., Lapini, G., 2012. Cell culture-derived influenza vaccines from Vero cells: a new horizon for vaccine production. *Expert Rev. Vaccines* 11, 587–594.
- Perdue, M.L., Arnold, F., Li, S., Donabedian, A., Cioce, V., Warf, T., Huebner, R., 2011. The future of cell culture-based influenza vaccine production. *Expert Rev. Vaccines* 10, 1183–1194.
- Salk, J.E., Bazeley, P.L., Bennett, B.L., Krech, U., Lewis, L.J., Ward, E.N., Youngner, J.S., 1954. Studies in human subjects on active immunization against poliomyelitis. II. A practical means for inducing and maintaining antibody formation. *Am. J. Public Health Nations Health* 44, 994–1009.