Original Article

Recombinant scFv inhibit Newcastle disease virus *in vitro* infection

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Abstract

Newcastle disease (ND) is an avian respiratory disease caused by the Newcastle disease virus (NDV) and is prevalent worldwide but lacks a reliable treatment to surmount it. The single-chain fragment variable (scFv) antibody is the smallest functional unit in an immunoglobulin retaining antigen-binding activities. Previous studies have demonstrated that by acting against the NDV phosphoprotein, scFv is capable of neutralizing the virus in infected cells. In this study, two anti-NDV scFv expressing cassettes driven by CAG promoter and EF1α promoter, respectively, were constructed using the piggyBac transposon plasmid backbone. The recombinant plasmids were transfected into DF-1 and 293T cells and four stable cell lines CAG-scFv-DF-1, EF1α-scFv-DF-1, CAG-scFv-293T and EF1α-scFv-293T were established after puromycin screening. RT-PCR results confirmed the presence of anti-NDV scFv mRNA in all four cell lines. Western blot results showed that scFv was detected in the culture supernatant of the CAG-scFv-DF-1 and EF1α-scFv-DF-1 lines, indicating that the scFv antibody was secretory after expression in the cells. Cytopathic effect assay showed that cells expressing scFv were more resistant to NDV F48E9 than those of control when the virus titer was not higher than 60×TCID50. Results in this study offer information to the generation of transgenic chickens resistant to NDV infection and for production of neutralizing antibodies against NDV.

Keywords: NDV, Single-chain fragment variable, Transgenic, PiggyBac transposon system

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Introduction

Newcastle disease (ND) is a highly contagious and acute septicemic poultry disease caused by the Newcastle disease virus (NDV) and is characterized by respiratory mucosa, gastrointestinal mucosa hemorrhage and nervous symptoms (Florman 1947; Ginsberg 1951). NDV is a member of the *Paramyxoviridae* family, which contains a single negative-stranded RNA genome of approximately 15 kb coding for six proteins including nucleoprotein, phosphoprotein, matrix protein, fusion protein, hemagglutinin-neuraminidase protein and RNA-dependent RNA polymerase (Seal *et al.* 2000). The RNA is encapsulated by nucleoprotein to form the RNA-N complex, which interacts with phosphoprotein and RNA-dependent RNA polymerase to constitute the full transcriptive complex (Hamaguchi *et al.* 1983). Phosphoprotein can interact with itself and nucleoprotein at the site of 247-291 aa. It acts as a chaperone of nucleoprotein and regulates the switch from transcription to replication(Chambers & Samson 1982; Jahanshiri *et al.* 2005). Currently, vaccines have been extensively used to prevent ND in the poultry industry. The most widely used efficacious live vaccines are based on low virulence viruses including Hitchner B1 (Hitchner & Johnson 1948; Chambers & Samson 1982) and LaSota strain (Goldhaft 1980). It provides limited protection for birds attacked by diverse types of NDV.

Several recombinant vaccines, such as Poxvirus-type vector expressing the NDV fusion protein or hemagglutinin-neuraminidase protein vaccine (Meulemans *et al.* 1988; Nishino *et al.* 1991), also provide protection against ND. However, vaccines offered the protective effect to avian before the viral infection. For a bird flock that has an outbreak of Newcastle disease there's little that can be done. Therefore, the development of a feasible treatment is essential to overcome the ND that occurs in the poultry industry.

Single-chain fragment variable (scFv) antibodies are the smallest immunoglobulin molecules composed of variable regions of heavy (V_H) and light (V_L) chains linked by a flexible peptide. The scFv antibodies have low molecular weights and low immunogenicity but high penetrability. Anti-HIV-1 human scFv monoclonal has cross neutralizing function and could be a useful candidate for designing immunogen and passive immunotherapy (Nishino *et al.* 1991; Khan *et al.* 2017). Bovine scFv antibody expressed in *E. coli*.can inhibit bovine herpesvirus-1 infectivity by targeting viral glycoprotein D and decreases the number of viral plaques by blocking viral attachment to MDBK cells (Xu *et al.* 2017). Additionally, transgenic animals expressing the scFv antibody are also powerful tools for controlling virus disease. For instance, transgenic chickens expressing the cytosolic 3D8 scFv protein are more resistant to AIV infection (Byun *et al.* 2017). In recent studies the methods of preparation and identification of scFv antibody against NDV phosphoprotein have been reported and scFv antibodies with a neutralizing effect against NDV F48E9 strain have been identified, which will be useful for diagnosis and therapy of ND (Li *et al.* 2014a; Li *et al.* 2014b).

The piggyBac transposon is a DNA transposon first discovered in the cabbage looper moth *Trichoplusia ni* (Cary *et al.* 1989; Prak & Jr 2000) with flexible and high transposition activity which can integrate a large cargo size of exogenous sequence and maintain long-term gene expression (Ding *et al.* 2005; Nakanishi *et al.* 2010). The excision of the piggyBac transposable element is very precise and can bypass DNA synthesis during the cut and paste transposition, leaving no footprint in the host genome (Elick *et al.* 1996b; Wilson *et al.* 2007a; Mitra *et al.* 2008). piggyBac induced transposition has proven to be feasible in primordial germ cells (PGCs) and developed as an efficient tool for the construction of transgenic chickens (Lu *et al.* 2009; Liu *et al.* 2012; Macdonald *et al.* 2012; Park & Han 2012).

The scFv antibodies have been reported and have demonstrated the efficacy in mammals (Ahmad *et al.* 2012; Gupta & Shukla 2017; Khan *et al.* 2017). However, research of avian scFv antibodies is largely unknown. This study attempted to explore the resistance of chicken scFv antibodies to NDV. The expressing cassettes of anti-NDV phosphoprotein scFv were constructed in piggyBac transposon backbone and then transfected into chicken DF-1 cells and human HEK-293T cells. The scFv antibody purified from the supernatant from the transfected cells demonstrated the high inhibitory effect of NDV. Our study lays a foundation for production of scFv NDV infection in transgenic chickens.

Materials and Methods

Cells and virus: DF-1 cells and HEK-293T cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) at 37 ℃ and 5% CO_2. Newcastle disease virus F48E9 strain was purchased from the China Institute of Veterinary Drugs Control (Beijing, China) and propagated in DF-1 cells.

Plasmids construction and transfection: The piggyBac transposon and the piggyBac transposase plasmids were purchased from System Bioscience (Palo Alto USA). The nucleotide sequence of scFv against the P protein of NDV was synthesized according to previous reports and inserted in the plasmid. The expression cassette was driven by the promotor of CAG or EF1α. A His tag was added to scFv to facilitate the purification of scFv in the future (Fig.1).

DF-1 cells and HEK-293T cells were seeded into 24-well plates 24 h prior to transfection. In this study, 1.44×104 cells were in each well. 50μl Opti-MEM® Medium was utilized as the solvent for the dilution of recombinant plasmid (1.0 μg) and the mPB helper plasmid (0.5 μg). The final plasmid concentration was 2131ng/μl for transfection. Both cell lines were co-transfected with transposon and transposase plasmids at 1:1 ratio using Lipofectamin 2000 (Invitrogen, Carlsbad, CA, USA) according to the user's manual. Forty-eight hours later, cells were cultured in 7% FBS DMEM containing 1μg/mL puromycin. The screening continued for three passages to obtain a stable transgene cell line.

RNA isolation and RT-PCR: The total RNA of cell samples was isolated using a RNAprep Pure Cell/Bacteria Kit (Tiangen, Beijing, China) according to the user's manual. Total cDNA was synthesized using the FastQuant cDNA RT kit (Tiangen, Beijing, China) according to the user's manual. PCR was performed using 2×Taq PCR Master Mix (Tiangen, Beijing, China) and the primers used in this study are listed in Table 1. Equivalent cells in 4 cell lines were collected for RNA extraction and Reverse transcription. The obtained cDNA was applied to conduct the PCR experiment, and the products were identified by electrophoresis on 2% agarose gels. GelDoc XR System - (BIO-RAD, American) was used to take photos for analysis. The scFv band width and brightness present the scFv density. In this study, a 20 µl reaction system was used for the target fragment amplification for, in total, 30 cycles. The PCR products were separated by electrophoresis on 2% agarose gel.

Figure 1 Plasmid of scFv anti-NDV

Table 1 The primer sequences used for real-time PCR to detect the expression of scFv mRNA

Primer name	Sequences (5'-3')	Annealing temperature (℃)	Product Size(bp)
scFv1_F	CAGGTGGTCGTTGGTATC	54.5	256
scFv1_R	GGTCTGTTGGTGTTAGCA		
scFv2_F	CTATGCTAACACCAACAGAC	53.7	197
scFv2_R	GATGATGATGATGATGTAGGA		
GAPDH_F	TGCCCAGAACATCATCCCA	59	295
GAPDH_R	GCCAGCACCCGCATCAAAG		

Western blotting analysis: The cell lysates and supernatant from the culture of transfected cells were collected for Western blot analysis after cell culture for 4 days. The supernatant was concentrated via the trichloroacetic acid (TCA) method and the cells were lysed with RIPA lysis buffer containing protease inhibitor. Each cell line was cultured in 100 mm culture dish until full and replaced by a new medium for 4 days incubation, then 9.0 mL supernatant was collected into 15 mL centrifuge tube and then 1.0 mL TCA added for supernatant dealing. On the other hand, the cells were collected by 90% scale, washed by PBS three times and then lysed by RIPA lysis buffer 1.0 mL per dish (Size:100 mm). Based on the collection of protein of supernatant and cells, Western blotting experiment was conducted to determine scFv expression and secretion. SDS-PAGE gel electrophoresis and transfer and the subsequent immuno-detection were carried out following the exact procedures as described previously (Khan *et al.* 2017). The scFv expression was detected by mouse anti-His primary antibody (1:500, ABGENT, Suzhou, China) and goat anti-mouse HRP-conjugated secondary antibody (1:10000, Thermo fisher, Shanghai, China). As a quality control, cells and supernatant of the DF-1 cells, HEK-293T cell lines were also collected.

Virus $TCID_{50}$ assay and cytopathic effect (CPE): The DF-1 cells were seeded in a 96-well plate 24 h prior to viral infection. The virus F48E9 was diluted in 10-fold series from 10^{-5} to 10^{-15} in DMEM. The diluted virus was inoculated into the culture in a 96-well plate (100 µL per well) and incubated at 37 ℃ for 2 h. DF-1 cells inoculated with DMEM served as blank controls. After incubation, culture medium was replaced by fresh DMEM supplemented with 2% FBS and observation was done every 12 h for 5 days to examine CPE. $TCID_{50}$ was calculated according to the Reed-Muench formula.

Results

Construction of anti-NDV scFv expressing plasmids: To obtain the scFv protein expression in chicken cells, the CMV promoter was replaced by a CAG promoter and EF1α promoter, respectively. For convenience and accuracy, the CAG-scFv sequence (Fig.6) was chemically synthesized. Both the CAG-scFv sequence and the PB513B-1 plasmid were cleaved at SpeI and NotI sites, to replace the CMV promoter with the CAG-scFv sequence. The first recombinant plasmid was named as PB513B-CAG-scFv (Fig.7 A). The EF1α promoter sequence was amplified from PB513B-1 plasmid with SpeI and EcoRI sites introduced into the promoter. The EF1α promoter sequence and PB513B-CAG-scFv plasmid were both cleaved by SpeI and EcoRI, to replace the CAG promoter with EF1α promoter. The second recombinant plasmid was named as PB513B-EF1α-scFv (Fig.7 B). The sequences of the recombinant plasmids were confirmed by sequencing (data not shown).

ScFv expression in transgenic cell lines: PB513B-CAG-scFv or PB513B-EF1α-scFv, and pPBase, were co-transfected into DF-1 cells and HEK-293T cells for 48 h., respectively. To eliminate green fluorescent protein (GFP) negative cells, transfected cells were treated with 1.0 µg/mL puromycin for three generations. Ultimately, four GFP expressing cell lines were screened. These cell lines were named as CAG-scFv-DF-1 cell, EF1α-scFv-DF-1 cell, CAG-scFv-293T cell and EF1α-scFv-293T cell, respectively (Fig.2 A-D). These cell lines expressed GFP after three passages,

indicating that the piggyBac transposon had been stably integrated into in DF-1 cells and HEK-293T cells.

The four above mentioned GFP expressing scFv cell lines were harvested when they were confluent in monolayer culture. PCR was performed to detect their scFv mRNA expression. Gel electrophoresis showed that the fragment of scFv cDNA was detected in four scFv cell lines, as well as in the two positive control plasmids but not in the blank controls and the negative controls (Fig.3, upper panel). The GAPDH for DF-1 cells and β-actin for HEK-293T cells were used to normalize the cDNA loading (Fig.3, lower panel). The density of scFv band in EF1α-scFv-DF-1 cells was higher than that of CAG-scFv-DF-1 cells but CAG-scFv-293T cells and EF1α-scFv-293T cells were similar about the scFv mRNA level. The expression and localization of scFv protein in these cell lines were further analyzed. Western blot result showed that the scFv protein was not detected in the cell lyates of all four transgenic cell lines. However, it could be detected in the supernatant of CAG-scFv-DF-1 cells and EF1α-scFv-DF-1 cells (Fig.4). The expression level of scFv protein in the EF1α-scFv-DF-1 cells supernatant was significantly higher than those in CAG-scFv-DF-1 cells supernatant.

The antiviral effect of scFv DF-1 transgenic cells against NDV: The titer of the NDV F48E9 strain used in this study was measured on parental DF-1 cells. The $TCID_{50}$ of F48E9 strain was $10^{-8.76}$/0.1 mL. CAG-scFv-DF-1 cells, EF1α-scFv-DF-1 cells and DF-1 cells were infected with 6×$TCID_{50}$, 60×$TCID_{50}$ and 600×$TCID_{50}$ NDV F48E9 strain, respectively. Then the numbers of wells presenting CPE were counted and the antiviral effect of each cell line against F48E9 strain was evaluated. After infection with 6×$TCID_{50}$ NDV, CPE was observed in 22 out of 24 wells in CAG-scFv-DF-1 cells, which was similar to DF-1 cells (CPE occurred in 14 out of 19 wells). After infection with 60×$TCID_{50}$ NDV, CPE was observed in all 24 wells of CAG-scFv-DF-1 cells and DF-1 cells. However, after infection with 6×$TCID_{50}$ and 60×$TCID_{50}$ NDV, CPE was only observed in 2 out of 24 wells and 6 out of 24 wells of EF1α-scFv-DF-1 cells, which were significantly lower than that in CAG-scFv-DF-1 cells and DF-1 cells, respectively. After infection with 600×$TCID_{50}$ NDV, CPE was observed in all wells in three cell lines (Fig.5). These results suggested that the EF1α-scFv-DF-1 cells were more resistant to the NDV infection than CAG-scFv-DF-1 cells and DF-1 cells. Therefore, the scFv protein against P protein of NDV under the regulation of EF1α promoter in piggyBac transposon in vivo appeared to be a potential strategy to prevent and control NDV infection.

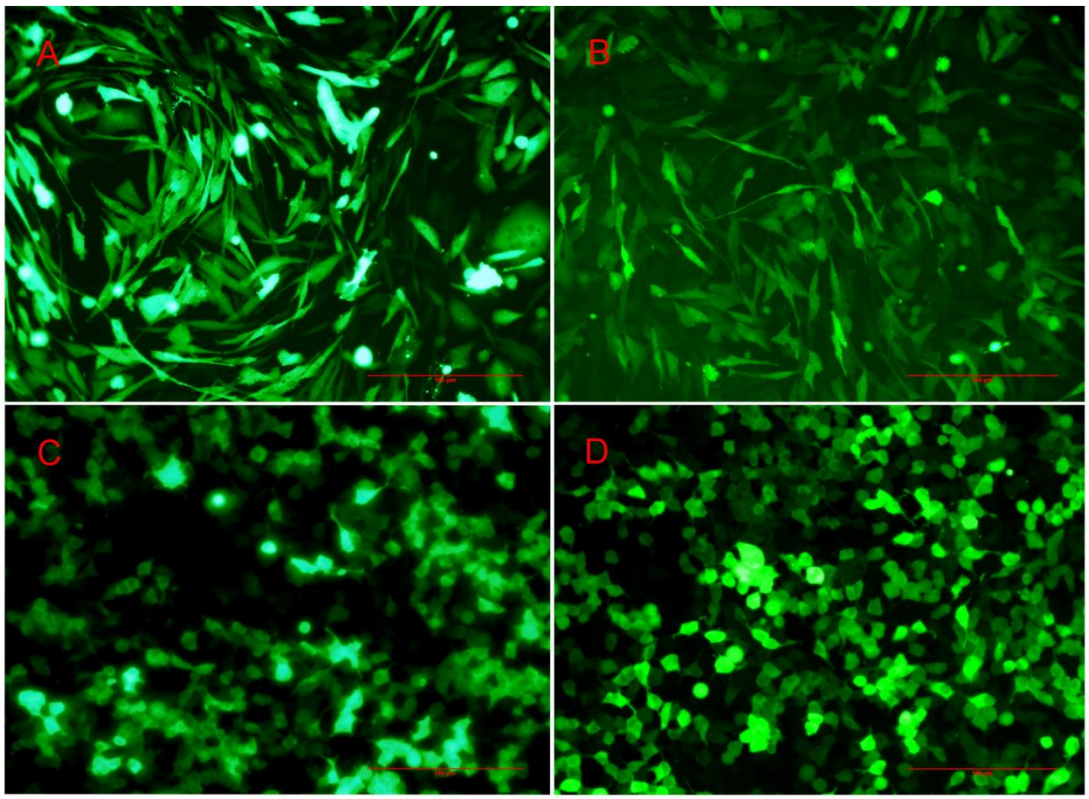

Figure 2 Stable cell line expressing GFP (scale bar = 200 μm). (A) CAG-scFv-DF-1 cell line; EF1α-scFv-DF-1 cell line; CAG-scFv-293T cell line; EF1α-scFv-293T cell line.

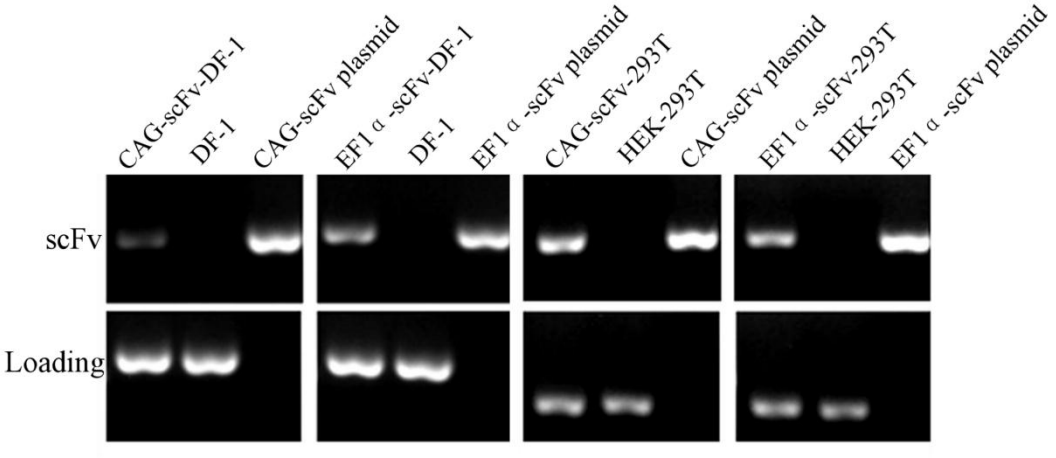

Figure 3 Detection of scFv expression using RT-PCR

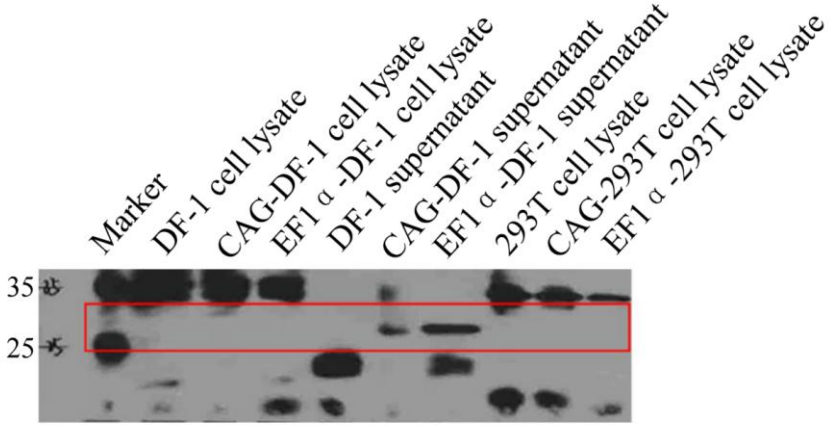

Figure 4 Detection of scFv in cell lysate and supernatant

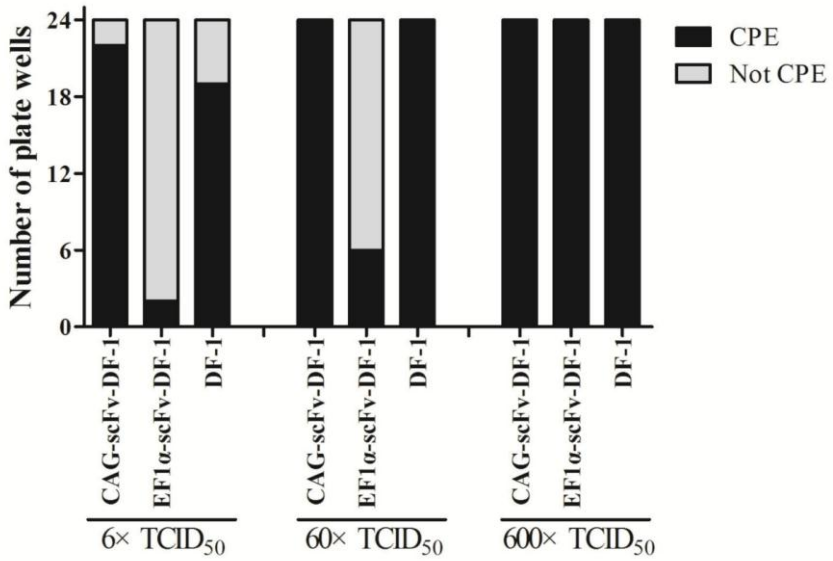

Figure 5 The antiviral effect of scFv transgenic cells on NDV.

ACTAGTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGA
CGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCAC
TTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGC
ATTATGCCCAGTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATC***GAATTC***GCCGCC
ACCATGGCCTGGGCCCCTCTCCTCCTCGCGGTGCTCGCCCACACCTCAGGTTCCCTGGTCCAGGCAGCAATGGCCGT
GACGTTGGACGAGTCCGGGGGCGGCCTCCAGACGCCCGGAGGCGCGCTCAGCCTCGTCTGCAAGGGCTCCGGGTTC
ACCTTCAGCAGCCATGGCATGGGCTGGGTGCGACAGGCGCCCGGCAAGGGGCTGGAATACGTCGCAGCTATTAGCA
GCACTGGGACTTACACGAACTACGGGGCGGCGGTGAAGGGCCGTGCCACCATCTCGAGGGACAACGGGCAGAGCA
CAGTGAGGCTGCAGCTGAGCAACCTCAGGGCTGAGGACACCGGCACCTACTACTGCGCCAAAAGTGCAGGTGGTC
GTTGGTATCCATATAATATCGACGCATGGGGCCACGGGACCGAAGTCATCGTCTCCTCC**GGTGGAGGCGGTTCAGG**
CGGAGGTGGCTCTGGCGGTGGCGGATCGCTGACTCAGCCGTCCTCGGTGTCAGCAAACCTGGGAGGAACCGTCGAG
ATCACCTGCTCCGGGGGTACCAGCAACTATGGCTGGTACCAGCAGAAGGCACCTGGCAGTGCCCCTGTCACTGTGA
TCTATGCTAACACCAACAGACCCTCGAACATCCCTTCACGATTCTCCGGTTCCACATCCGGCAGAACCACAAACAC
ATTAACCATCACTGGGGTCCAAGCCGAGGACGAGGCTGTCTATTTCTGTGGTGCCTACGACGGGGGAACTTCTACA
TTTGGGGCCGGGACAACCCTGACCGTCCTACATCATCATCATCATCATTAATGA***GCGGCCGC***

Figure 6 The sequence of CAG-scFv

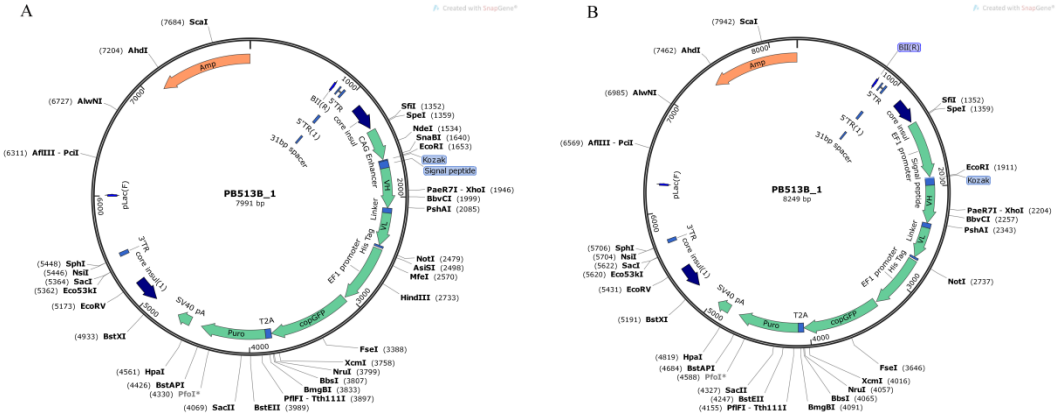

Figure 7 The profile of experiment plasmid. (A)The profile of PB513B-CAG-scFv plasmid; (B) The profile of PB513B-EF1α-scFv plasmid.

Discussion

In the present study, transgenic cell lines that expressed the scFv protein for against the NDV P protein were generated using the piggyBac transposon. The generated scFV were demonstrated and evaluated the antiviral effect of the transgenic cell lines against NDV.

The scFv antibody is a genetically engineered antibody widely used in biopharmaceutical, immunodetection, environmental protection, clinical diagnosis and therapy (Griffiths & Duncan 1998; Ahmad *et al.* 2012; Gupta & Shukla 2017). Previous study has demonstrated that the linker (Gly4Ser)3 between VH and VL increased the efficiency of scFv protein expression and folding and maintained the stability, affinity and specificity of scFv (Krebber *et al.* 1997). Therefore, in this study, a widely used linker was inserted between VH and VL to evaluate the efficacy of scFv protein (Krebber *et al.* 1997). The scFv antibody could be produced in various systems including phage-displayed synthetic scFv library (McCafferty *et al.* 1990; Griffiths & Duncan 1998), *Escherichia coli* (*E. coli.*) (Jurado *et al.* 2002), eukaryotic cells (Cattaneo & Biocca 1999) and transgenic animal models (Byun *et al.* 2017). Recently, the scFv antibody against the NDV P protein expressed in *E. coli* reported the ability to neutralize NDV in BHK-21 cells culture (Li *et al.* 2014a; Li *et al.* 2014b). However, the anti-NDV effect of this scFv antibody expressed in eukaryotic system remains to be evaluated in the susceptible chicken cells. Transgenic chickens expressing scFv antibody are potential models to evaluate the function of scFv antibody.

It was reported that the exogenous protein expression was reduced over generations in transgenic chickens (Kamihira *et al.* 2005; Byun *et al.* 2017). Moreover, the use of viral vector in transgenic animals might raise safety issues. Based on these considerations, a safer strategy to express anti-NDV scFv protein in transgenic cells and chickens is required. In this research, we used the piggyBac transposon to express the scFv protein in chicken DF-1 cells and evaluated its anti-NDV effect. PiggyBac transposon can efficiently integrate transgenes into host cell genomes for transgenesis and germline mutagenesis with extreme specificity for TTAA target sites (Fraser *et al.* 1996) and precise excision (Elick *et al.* 1996a). Without overproduction inhibition (Wilson *et al.* 2007b), as a non-viral vector, piggyBac transposon is safer and has a larger payload capacity than retroviral vectors, capable of delivering 9.1-14.3 kb sequences without a significant reduction of the transposition frequency (Ding *et al.* 2005). Therefore, it has been used in a broad range of hosts including insects, mammals and poultry. Our result showed that the transfection efficiency of piggyBac in DF-1 cells (20.5%) was similar to that of HEK-293T cells (25.6%) through Lipofectamin 2000. This indicated that both the chicken

and human cells were suitable for piggyBac transposon. We constructed two piggyBac recombinant plasmids, PB513B-CAG-scFv and PB513B-EF1α-scFv. Both plasmids expressed the GFP reporter, which allowed us to evaluate the transfection efficiency and to screen the scFv-expressing cells in DF-1 cells and HEK-293T cells. Ultimately, we screened out four transgenic cell lines expressing GFP protein and examined scFv mRNA as well as protein expression in these cell lines.

In this study, scFv expression in transgenic cell lines was driven by either CAG or EF1α promoter. CAG promoter is composed of a chimeric CMV early enhancer with a chicken β actin transcription start site and a rabbit β globin intron. CAG promoter is ubiquitously used in a variety of cells including somatic cells and stem cells. Gene expression driven by the CAG promoter is long-lasting in embryonic stem cells (ESCs) and mice. In contrast, gene expression driven by viral promoters such as cytomegalovirus (CMV) and SV40 promoters is often silenced (Lutzko *et al*. 2003; Seo *et al.* 2010; Grozdanov & MacDonald 2015a; Yang *et al*. 2017). EF1α promoter is another well-characterized non-viral promoter. EF1α promoter increases the transfection efficiency, the transgene expression, the proportion of expression-positive clones and the copy number of episomal vector in long-term culture compared with CMV and CAG promoter (Grozdanov & Macdonald 2015b; Wang *et al.* 2017a; Wang *et al.* 2017b). To optimize scFv expression in transgenic cells, we compared the transcriptional efficiency between CAG promoter and EF1α promoter and found that both of the mRNA and protein levels of the scFv gene under the regulation of EF1α promoter were higher than that of CAG promoter, which was consistent with the results of Wang et al. (Wang *et al.* 2017a; Wang *et al*. 2017b). This probably indicated that the transcriptional activity of EF1α promoter was higher than the CAG promoter in DF-1 cells, but not in HEK-293T cells. The transcriptional efficiency of a promoter is determined by many factors including genomic *cis*-acting sequences, cell lines, type of vector and transcription factor-binding site. Our results indicated that EF1α promoter was more suitable for transgenesis than CAG promoter in chicken DF-1 cells. Therefore, we speculate that EF1α promoter in the piggyBac transposon backbone is also useful for generation of chicken PGC cell lines.

The scFv mRNA could be detected in all four transgenic cell lines. However, scFv protein expression was not detected in any cell lysate of these cell lines and was only detected in the supernatant of CAG-scFv-DF-1 and EF1α-scFv-DF-1 cell lines. This result could probably be explained by the secretory property of scFv protein. Guided by the chicken signal peptide, more scFv protein produced in cells might have been secreted into the supernatant by 96 h. Therefore, the amount of intracellular scFv protein might be too low to be detected. As HEK-293T cells cultured in low or none serum DMEM will shed after 2 h until death, we were unable to culture CAG-scFv-293T cells and EF1α-scFv-293T cells for 96 h in low serum DMEM and to collect their supernatant for protein detection. Therefore, only supernatant from CAG-scFv-DF-1 cells and EF1α-scFv-DF-1 cells were analyzed in this study and our result show that scFv protein was present in the supernatant of these two transgenic DF-1 cell lines. This demonstrates that guided by the chicken signal peptide, scFv proteins were secreted out of the cells and the translation activity of EF1α promoter was stronger than that of CAG promoter in DF-1 cells. In future studies it would also be informative to analyze the supernatant of CAG-scFv-293T cells and EF1α-scFv-293T cells what were only cultured for a short period, to better evaluate scFv protein expression in these cell lines.

We compared the antiviral effects against NDV F48E9 strain between CAG-scFv-DF-1 cells and EF1α-scFv-DF-1 cells. EF1α-scFv-DF-1 cells displayed a stronger antiviral effect than CAG-scFv-DF-1 cells, which was consistent with the different scFv protein expression levels in the supernatant of these two cell lines. Current research has revealed that monovalent antigen-binding fragments and scFv antibodies demonstrate similar binding specificity and affinity to parental anti-Occludin monoclonal antibodies, as well as Fab fragments and scFv antibodies inhibit *in vitro* HCV infection (Shimizu *et al*. 2021). This indicates that the dosage of scFv protein might be a critical factor for inhibiting NDV growth. Moreover, the scFv protein against the P protein of NDV using the piggyBac transposon in vivo is able to inhibit NDV F48E9 infection, which coincides with the results of Li *et al* (Li *et al.* 2014a; Li *et al.* 2014b). . In conclusion, we successfully generated the scFv transgenic cell lines against NDV infection using piggyBac transposon and we showed that the scFv protein expressed in DF-1 cells inhibited the NDV infection. We also showed that EF1α promoter was more efficient than CAG promoter for scFv expression in DF-1 cells. To our knowledge, this is the first report in which piggyBac transposon is utilized to express anti-NDV scFv antibody in a chicken cell line. These results provideda strong theoretical basis for production of scFv transgenic chickens using piggyBac transposon against NDV infection.

Competing interests: The authors declare no competing financial interests.

Ethics approval: All materials and animal cell studies were approved by the Committee on Experimental Animal Management of Guangxi University.

Consent for publication: All authors agree to submit this manuscript entitled "*Recombinant scFv Inhibit Newcastle Disease Virus Infection*" in The Thai Journal of Veterinary Medicine.

Availability of data and material: All the data and material in the manuscript are available. Neither the entire paper nor any part of its content has been submitted or published in any other journal.

Author contributions: Yangqing Lu and Sanfeng Peng conceived and designed the experiments. Sanfeng Peng, Ying He, Dongyang Chen, Long Xie, Li Wang and Xueliang Zhou performed the experiments. Sanfeng Peng and Lintian Yu analyzed and interpreted the data. Sanfeng Peng, Lintian Yu, Ying He and Yangqing Lu drafted and revised the manuscript.

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