Preparation and bioactivity of anti-Newcastle disease virus-phosphoprotein cytoplasmic transduction peptide antibody

Benqiang Li (libenqiang2007@163.com)
Sveriges Lantbruksuniversitet Veterinarmedicin och husdjursvetenskap
Man Wang
Institute of animal science and veterinary medicine
Jianguo Zhu
Institute of animal science and veterinary medicine

Research article

Keywords: Newcastle disease virus, phosphoprotein, cell-penetrating peptide, antiviral activity

Posted Date: January 21st, 2020

DOI: https://doi.org/10.21203/rs.2.21452/v1

Abstract

On the basis of cell-penetrating peptide's character that it can penetrate cytomembrane and transfer macromolecular protein to cytoplasm so to play biological function, we took the experiments. The fuse penetrating peptide our experiment adoptted is HIV-TAT derived fragment-CTP512, with good transmember effect and distinct cytoplasm-position. In this chapter, the research of transmembrane character was processed first. According to the tests on trans- member protein with different concentrations, the best trans-member concentration is 3μM. Afterwards, we found that the location of trans-member antibody is overlapping with phosphoprotein using indirect immunofluorescence test analysis. According to MTT test, there is no significant difference between CTP fusion protein and control on cell proliferation and viability. TCID50 test was used to detect the protective effect of trans-member antibody on cell. Result showed that trans- member antibody has significant cell protection effect compared to the control in the order: ZL.103>ZL.17>Control. Fluorogenic quantitative PCR result showed that trans- member antibody can disturb the duplication and transcription of Newcastle disease virus. This results not only paved a good way to research the transport of disease related protein, but also provide a splendid tool on protein function research.

Backgroud

Current research show that, whether in medicine or veterinary field, the efficient system conducting antibody to animal tissue cell is in shortage, which restricts the research and development of antibody as anti-virus drug[1, 2]. Previously, most carriers delivering antibody into cell is virus carrier, however, the problem of security and immunity are not be solved properly. At present, most research mainly made antibody coding gene recombinant to common expressed eukaryotic carrier. However, these carriers do not possess the infecting ability to cell, they need assistant method to be inoculated into cell, such as lipofectamine carrying coding plasmid of antibody so to infect cell, or use particular inject device to guide antibody into cell[3, 4]. However, these methods are all infection in vivo, it's difficulty to take effect in living animal tissue cells. So the proper delivery system is one of the key technologies in antiviral antibody research.

Recently, in medical domain, the appearance of cell-penetrating peptide-a new type transbody threw light on the research of antibody[5, 6]. The mechanism of the entrance to cell of cell-penetrating peptide is unclear, however, its appearance supply a good method to biotherapy and scientific research. Cell-penetrating peptide is a kind of micro-molecule peptide which able to carry external material into cell[7]. So far, it is reported TAT, VP22, MPG, Pep-1 etc. as cell-penetrating peptide[8]. Among them HIV-TAT and its derived fragment are the most popular used cell-penetrating peptide[9, 10]. However, because of the nuclear localization of TAT, which function fusing to protein is influenced[11, 12]. By transforming the order and structure of HIV-TAT's amino acid, Kim built a new type cytoplasm transmit peptide(CTP), which own good cell member-penetrating character and marked cytoplasm-position feature[13]. As we know, Newcastle disease virus phosphoprotein is located in cytoplasm, so it is advisable to research the interaction of trans-member antibody and phosphoprotein.

In this paper, we built chicken original cytoplasm-expressed gene infused with CTP sequence, which might conduct trans-member transport of antibody in cell. After penetrating of cytomembrane, the antibody will realize the cytoplasm-position in accordance with the location sequence. (So to build valid delivery system of trans-member antibody).

Methods

Construction of cytoplasmic transduction peptide-single chain antibody fusion protein

Anti-phosphoprotein scFvs have been cloned from the spleen of immunized chicken in our laboratory, referred to in our publication Biologicals[14]. The CTP was added to 3' terminal. The forward primer was designed as 5'-CGGAATTCGCCGTGACGTTGGAC-3' with *Eco*R I; and the reverse primer was 5'-GAGTCATTCTGCGGCCGCACGGCGACGCTGGCGACGTTTCTTACGACCGTATAGGACGGTCAGGGTTGTCCC-3' with *Not* I and CTP. The anti-P CTP-scFv gene was amplified by PCR. after digested with *Eco*R I and *Not* I, the CTP-scFv gene was subcloned into the pET28a(+). In this way, the recombinant expression plasmid pET28a-CTP-scFv was obtained.

Stable expression of pET28a-scFv-CTP

Trans1-Blue transfected with the recombinant plasmid pET28a-scFv-CTP was propagated at 37°C in Luria Bertani (LB) medium supplemented with 100 mg/ml Kanamycin until the bacterium reached logarithmic growth phase (at OD600=0.7), and then induced by addition of 1mM isopropyl b-d-thiogalactopyranoside. Purification of the His-tagged phosphoprotein was carried out in accordance with the manufacturer's procedures for Ni+–NTA resin-packed columns. The final protein concentration was determined by the BCA kit.

The best contration of transduce of pET28a-scFv-CTP fusion protein

BHK21 cell were passaged (and immigrated) into 6 pore plate. That is : Cells in 100ml cell culture bottle were washed twice using DMEM, after 1min's digestion using tyrisin, cells were blown and suspended in 4mLDMEM(10% FBS with penicillin-streptomycin), and were immigrated into 6 pore plate (0.6ml cell suspension per pore), then were cultured in incubator overnight. When cell's density reached to 80%, cells were treated with protein in different concentrations respectively as following: 1) DMEM (without FBS) 1mL; 2) DMEM(without FBS) 1mL with scFv in final concentration 3uM; 3) DMEM(without FBS) 1mL with CTP- scFv in final concentration 1uM; 4) DMEM(without FBS) 1mL with CTP- scFv in final concentration 3uM; 5) DMEM(without FBS) 1mL with CTP-scFv in final concentration 4uM. 2h culture later, cells were washed twice in DMEM(or: after 3h incubating, cells were washed twice in PBS, then replaced new medium for another 24h culture. Washed three times in PBS and then observed the expression). After 1min digestion using tyrisin, cells were blown slightly and suspended in 1mLDMEM(10% FBS with penicillin-streptomycin). Suspension were collected in EP tube and taken Western blotting to analysis transmembrane effect.

Cytoplasmic localization of CTP-scFv

pET28a-scFv-CTP fusion protein was transduced into BHK21 cells as described above. After 24h, fixed with 4 % paraformaldehyde for 30 min and permeated with 0.1 % Triton-100 for 10 min. Cells were incubated with primary antibody mouse anti-His tag mAb at a dilution of 1:50 for 2 h, followed by incubation with secondary antibody FITC conjugated goat anti-mouse IgG at 1:25 for 1h at room temperature. Cells were analyzed and photographed with Confocal Laser Scanning Microscope, Leica TCS-SP.

MTT test to detect CTP influence on cell viability

Cells were collected in logarithmic phase, and were made into 10^5/ml suspension using DMEM containing 10% FBS, then were inoculated in 96 culture plate. After 24h, pET28a-scFv-CTP and scFv were treated to cell with final concentration 0.5mg/ml, DMEM was contrast. Every sample owned 4 holes as parallel. Cells were cultured for in 37℃ and 5%CO2. After 24h, cells were treated with 20μl 5mg/ml MTT（soluted in PBS）, after incubating for 4h, supernatant were removed, and the culture plate was inverted on several layers filter papers so to wipe put supernatant sufficiently. Added DMSO 150 μl per hole, the cells were shaked in shaker for 30min. The OD value were detected in 570nm/490nm using microplate reader.

Virus infection

To assess the effect of anti-P CTP-scFv antibody on NDV production, BHK21 cells were stably transduced with pET28a-scFv-CTP fusion protein, respectively. Then infected with the virus at a multiplicity infection of 0.01, supernatants of cell cultures were harvested at 12, 24, 36 and 48h. serially diluted, and assayed for virus titer by TCID50 assay[15].

Reverse transcription and real-time PCR

Transducted BHK21 cells grew exponentially in 96-well plates on glass cover-slips and 24 post-transducted, infected with NDV at a multiplicity of infection of 0.1, 4h post-infected, culture medium was removed and cells were collected. Total RNA was isolated from lysed cells using Trizol reagent according to the procedures described by manufacturer. Reverse transcription was carried out by using reverse transcriptase in 20 μl reaction mixture, containing 200 ng of total RNA and specific primers for P-mRNA (5'-TTTTTTTTTTTTTTTTTT-3'), P-cRNA (5'-TGGTGATCAGCCATTCAGCGCAAGGC-3'), and P-vRNA (5'- TACCCAGCAGACCAGGGCGAATATG-3'), at 42 ℃ for 1 h. RT reaction mix was then used for real-time PCR using specific primers for P (sense 5'-CCTTTACAGACGCGGAGATTG-3' and antisense 5'-GTTTTGCCTTGTGGGATTGC-3') andβ-actin (sense 5'-GCATCCACGAAACTACATTCAACTC-3' and antisense 5'-CACTGTGTTGGCATAGAGGTCTTTG-3'), and SYBR green I dsDNA binding dye. Real-time PCR reaction was performed at 95 ℃ for 3 min for 1 cycle and then 94 ℃ for 45 s, 55 ℃ for 30 s and 72 ℃ for 1 min for 40 cycles. PCR products were measured with Rotor-Gene 2000 Real-time Cycler and analyzed with Rotor-gene software. Cycles times (Ct) were analyzed at a reader of 0.2 fluorescence unit. The duplicate cycle times were averaged and normalized to the cycle time of β-actin. All reactions were done in duplicate.

Results

Construction of pET28a-scFv-CTP

The product of RT-PCR coincided with what we preconceived. A desired PCR product of 783 bp was obtained after agarose gel electrophoresis analysis. Then the phophoprotein gene was digested by the double restriction enzyme and further cloned into pET28a(+) vector. The recombinant plasmid pET28a-P was successfully constructed, and the enzyme digestion analysis was identical with the expected results(Fig1. A).

Expression of anti-P pET28a-scFv-CTP

In order to obtain the purified pET28a-scFv-CTP fusion protein, the constructed prokaryotic expression plasmid of pET28a-scFv-CTP was transformed into Trans1-Blue and inducted by 1mM IPTG. A high level of expression protein was obtained. The expression fusion protein with molecular weight of about 33 KD was detected by SDS-

PAGE (Fig1. B). The recombinant protein was purified through Ni-chelating affinity chromatography, and the purity was above 85% by SDS-PAGE gel scan analysis (Fig1. C).

Trans-membrance activity of pET28a-scFv-CTP fusion protein

Immunofluorescence studies were carried out to identify transduced pET28a-scFv-CTP fusion proteins could penetrate in cells. The results showed the the purified pET28a-scFv-CTP fusion proteins have efficient trans-membrance activity and the best contration of transduce of pET28a-scFv-CTP fusion protein is 3μM

Subcellular localization of transducted ZL.17 and ZL.103

Cellular localization of fusion protein ZL.17 and ZL.103 were investigated. BHK21 cells were transducted with the purified fusion protein ZL.17 and ZL.103 carrying an His tag. Results showed the anti-P fusion protein ZL.17 and ZL.103 were distributed uniformly throughout the cell(in both cytoplasm and nucleus)(Fig 1.D).

pET28a -scFv-CTP fusion protein influence on cell viability

MTT assay was performed to reveal whether the fusion protein were harming to BHK21 cells. The result indicated that they were found no cytotoxic effect of pET28a-scFv-CTP fusion protein to BHK21 cells(Fig 2).

Anti-P pET28a-scFv-CTP fusion protein affected NDV F48E9 virus production

Stable transducted cells lines were then infected with the virus at a multiplicity infection of 0.01. 24 h, 36 h, and 48 h post-infection, supernatants of cell cultures were harvested, serially diluted, and assayed for virus titer using the $TCID_{50}$ method. Results revealed that virus titers were reduced in cell lines stably expressing all the three anti-P CTP intrabodies, in the order of: ZL.17> ZL.103>control when compared to that of empty vector transfected cells. These results suggested that virus production was potently inhibited by ZL.17 and ZL.103(Fig 3).

Anti-P pET28a-scFv-CTP inhibitied viral transcription and replication

In order to determine the effect of anti-p intrabodies on viral transcription and replication, we measured the production of three different forms of NDV RNA (mRNA, cRNA and vRNA), by using an approach described previously[16]. Total RNA was isolated at 4 h post-infection when sufficient viral RNA transcription and replication had occurred. Viral mRNA, cRNA, and vRNA were reverse transcribed using corresponding primers and the quantity of cDNA products measured by real-time PCR. Data from the real-time PCR showed that the levels of P-specific mRNA were significantly decreased in the presence of ZL.17 and ZL.103. The levels of P-specific cRNA were significantly inhibited by two anti-P CTP intrabodies. In addition, the P-specific vRNA production was also inhibited by two anti-P intrabodies in the order: ZL.17ZL.103Control(Fig 4). These results suggested that anti-P CTP intrabodies have inhibitory effects on the production of NDV mRNA, cRNA and vRNA.

Discussion

CTP is a kind of antibody protein with fusion expression of transport region[17, 18]. By means of/Via transmembrane function, CTP can conduct the transmembrane transport of antibody(expressed protein), and then located in cytoplasm[19, 20]. As this antibody is protein itself, it need not to be expressed in host cell, so the security is guaranteed in most extent. The consist of cell penetrating peptides is multiple, that it composes of 7–16 amino acid oligopeptides[8]. Generally, there is no or little homology in primary structure and secondary

structure of penetrating peptides, however, the common feature is that penetrating peptide is rich in amino acid residue, especially arginine, which has the interaction with the of negative pole in cell surface[21]. Cell penetrating peptide has low toxicity, and besides biomacromolecules such as polypeptide, protein and nucleic acid, it can carry moleculer drug, eikonogen and so on, hence, penetrating peptide is well used in cell research.

So far, it has not been reported that, by means of protein prokaryotic expression and purification technology, combined with cytoplasm transduce peptide fuse strategy, extrinsic protein peptide was transduced to the cytoplasm of BHK21 cell, and to bind with phosphoprotein specifically, so to inhibit the replication and transcription of phosphoprotein in Newcastle disease virus. It's a good tool in researching the transport of disease related protein, also a good method in studying protein function.

In past 20 years, the research of cell-penetrating peptide supplies a promising way in surmounting biological membrane barrier. In 1988, HIV-1Tat protein was firstly reported to be able to get through cytomembrane, soon afterwards, the antimmnpedia transcription protein in drosophila was reported to possess the similar transcription characters[22, 23]. The transduction ability of small peptide sequence to these transcription factor can be realized after the other macromolecule such as protein penetrating cell[24]. Many cell-penetrating peptides have been identified so far[25, 26]. These peptides become Trojan horse or protein transducing region at first, then turn to cell-penetrating peptide through internalization after reappraising, with showing endocytosis and assisting conduction, is a kind of primary internalization pathway[27]. Among them, the prior found protein-HIV-TAT and its derived fragment are well studied penetrating peptide. However, penetrating peptide has no specificity on tissues and cells, hence, the aiding method is needed in drug delivery system.

According to the character that the phosphoprotein in Newcastle disease virus participates in transcription and replication of RNA, and located in cytoplasm after infecting cell, the fused penetrating peptide our experiment adopted was HIV-TAT derived fragment-CTP512, with good transmembrane effect and distinct cytoplasm-position[10, 28, 29].

At present, the unsolved problem in administration route is how to transmit therapeutic biomacromolecule penetrating cytomembrane. The confronting problem is how to conquer the low bioavailability caused by biomacromolecule's size and water-wet behavior and the subsequent restriction on transmembrane. Now the problem become increasingly sharp, instead of biomacromolecule, more new drugs have to penetrate one or more lipid bilayer to reach the target spot. Many researches have testified the high efficiency of cell-penetrating peptide in carrying aim polypeptide in vitro. Schwarze has proved the transmembrane effect of cell-penetrating peptide in vitro that via the fusion of penetrating peptide to β-galactosidase, the protein transmit has realized by intraperitoneal injection in mouse[30]. Hence, cell-penetrating peptide is a promising tool in macromolecule delivery.

In this paper, we took the bioactivity analysis of transmembrane fusion protein we acquired. At first, whether pET28a-scFv-CTP protein can entrance BHK21 cell was detected. Result showed that pET28a-scFv-CTP fusion protein was succeed entrancing cell and being expression with concentration dependency that 3 μM is the optimum concentration. Cellular localization under fluorescence microscope found that transmembrane-antibody mainly distributed in cytoplasm, and was co-located with phosphoprotein of Newcastle disease virus. By means of TCID50 test analysis, the two transmembrane-antibodies were capable to protect cell from the infection of Newcastle disease virus. Fluorogenic quantitative PCR analysis found that, transmembrane-antibody can disturb

the production of cRNA, vRNA and mRNA which related to transcription and replication of virus, so to inhibit virus's transcription and replication.

Conclusion

Our experiment verified that, the transmembrane fusion antibodies we built not only develop efficient transmembrane character, but also conduct interest protein to target spot so to exert bioactivity. According to current report that penetrating peptide does not possess tissues and cells targeting property, and pharmacokinetics and body distribution studies have not been carried out, so there is certain distance from its clinical application[7, 31]. However, the results imply a new way in prevention and treatment of Newcastle disease, also provide basis to screening drug targeting internal structural protein of Newcastle disease virus.

Abbreviations

NDV：Newcastle disease virus；P：phosphoprotein；CTP：cytoplasmic transduction peptide

Declarations

Acknowledgement

We would like to thank Yuan Lin for his assistance with reagent preparation.

Authors' contributions

Jianguo Zhu conceived and designed the experiments. Benqiang Li performed the experiments. Man Wang and Fanqing Zhang contributed data analysis. All authors have read and approved the final version of manuscript.

Funding

This study was funded by the National Key Research and Development Program of China (No. 2017YFD0501102), Shanghai Agriculture Applied Technology Development Program of China (No.T20170111), and the Youth Talent Development Plan of Shanghai Municipal Agricultural System of China (No. 20160135).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Institutional Animal Care and use committee of Shanghai Academy of Agricultural Science.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Shanghai Key Laboratory of Veterinary Biotechnology, College of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, China

[2]Institute of Animal Science and Veterinary Medicine, Shanghai Academy of Agricultural Sciences, Shanghai 201106, China

[3]Shanghai Engineering Research Center of Breeding Pig, Shanghai 201106, China

References

1. Corte-Real S, Collins C, Aires da Silva F, Simas JP, Barbas CF, 3rd, Chang Y, Moore P, Goncalves J: Intrabodies targeting the Kaposi sarcoma-associated herpesvirus latency antigen inhibit viral persistence in lymphoma cells. *Blood* 2005, 106(12):3797-3802.
2. Guo C, Zhu Z, Yu P, Zhang X, Dong W, Wang X, Chen Y, Liu X: Inhibitory effect of iota-carrageenan on porcine reproductive and respiratory syndrome virus in vitro. *Antiviral therapy* 2019.
3. Gal-Tanamy M, Zemel R, Bachmatov L, Jangra RK, Shapira A, Villanueva RA, Yi M, Lemon SM, Benhar I, Tur-Kaspa R: Inhibition of protease-inhibitor-resistant hepatitis C virus replicons and infectious virus by intracellular intrabodies. *Antiviral research* 2010, 88(1):95-106.
4. Kvam E, Sierks MR, Shoemaker CB, Messer A: Physico-chemical determinants of soluble intrabody expression in mammalian cell cytoplasm. *Protein engineering, design & selection : PEDS* 2010, 23(6):489-498.
5. Ma WF, Chen HY, Du J, Tan Y, Cai SH: A novel recombinant protein TAT-GFP-KDEL with dual-function of penetrating cell membrane and locating at endoplasm reticulum. *Journal of drug targeting* 2009, 17(4):329-333.
6. Bolhassani A, Jafarzade BS, Mardani G: In vitro and in vivo delivery of therapeutic proteins using cell penetrating peptides. *Peptides* 2017, 87:50-63.
7. Cai SR, Xu G, Becker-Hapak M, Ma M, Dowdy SF, McLeod HL: The kinetics and tissue distribution of protein transduction in mice. *European journal of pharmaceutical sciences : official journal of the European Federation for Pharmaceutical Sciences* 2006, 27(4):311-319.
8. Rapoport M, Lorberboum-Galski H: TAT-based drug delivery system–new directions in protein delivery for new hopes? *Expert opinion on drug delivery* 2009, 6(5):453-463.
9. Kumar RV, Sinha VR: Newer insights into the drug delivery approaches of alpha-glucosidase inhibitors. *Expert opinion on drug delivery* 2012, 9(4):403-416.
10. Kadkhodayan S, Jafarzade BS, Sadat SM, Motevalli F, Agi E, Bolhassani A: Combination of cell penetrating peptides and heterologous DNA prime/protein boost strategy enhances immune responses against HIV-1 Nef antigen in BALB/c mouse model. *Immunology letters* 2017, 188:38-45.
11. Sandgren S, Cheng F, Belting M: Nuclear targeting of macromolecular polyanions by an HIV-Tat derived peptide. Role for cell-surface proteoglycans. *The Journal of biological chemistry* 2002, 277(41):38877-38883.
12. Ferrari A, Pellegrini V, Arcangeli C, Fittipaldi A, Giacca M, Beltram F: Caveolae-mediated internalization of extracellular HIV-1 tat fusion proteins visualized in real time. *Molecular therapy : the journal of the American*

Society of Gene Therapy 2003, 8(2):284-294.

13. Kim D, Jeon C, Kim JH, Kim MS, Yoon CH, Choi IS, Kim SH, Bae YS: Cytoplasmic transduction peptide (CTP): new approach for the delivery of biomolecules into cytoplasm in vitro and in vivo. *Experimental cell research* 2006, 312(8):1277-1288.
14. Li B, Ye J, Lin Y, Wang M, Jia R, Zhu J: Selection and characterization of single-chain recombinant antibodies against phosphoprotein of Newcastle disease virus. *Biologicals : journal of the International Association of Biological Standardization* 2014, 42(5):285-289.
15. Li J, Zhang Q, Wang T, Li C, Liang M, Li D: Tracking hantavirus nucleocapsid protein using intracellular antibodies. *Virology journal* 2010, 7:339.
16. Gee SC, Bate IM, Thomas TM, Rylatt DB: The purification of IgY from chicken egg yolk by preparative electrophoresis. *Protein expression and purification* 2003, 30(2):151-155.
17. Koren E, Apte A, Sawant RR, Grunwald J, Torchilin VP: Cell-penetrating TAT peptide in drug delivery systems: proteolytic stability requirements. *Drug delivery* 2011, 18(5):377-384.
18. Tripathi PP, Arami H, Banga I, Gupta J, Gandhi S: Cell penetrating peptides in preclinical and clinical cancer diagnosis and therapy. *Oncotarget* 2018, 9(98):37252-37267.
19. Foged C, Nielsen HM: Cell-penetrating peptides for drug delivery across membrane barriers. *Expert opinion on drug delivery* 2008, 5(1):105-117.
20. Bohmova E, Machova D, Pechar M, Pola R, Venclikova K, Janouskova O, Etrych T: Cell-penetrating peptides: a useful tool for the delivery of various cargoes into cells. *Physiological research* 2018, 67(Supplementum 2):S267-S279.
21. Futaki S, Suzuki T, Ohashi W, Yagami T, Tanaka S, Ueda K, Sugiura Y: Arginine-rich peptides. An abundant source of membrane-permeable peptides having potential as carriers for intracellular protein delivery. *The Journal of biological chemistry* 2001, 276(8):5836-5840.
22. Frankel AD, Pabo CO: Cellular uptake of the tat protein from human immunodeficiency virus. *Cell* 1988, 55(6):1189-1193.
23. Joliot A, Pernelle C, Deagostini-Bazin H, Prochiantz A: Antennapedia homeobox peptide regulates neural morphogenesis. *Proceedings of the National Academy of Sciences of the United States of America* 1991, 88(5):1864-1868.
24. Fawell S, Seery J, Daikh Y, Moore C, Chen LL, Pepinsky B, Barsoum J: Tat-mediated delivery of heterologous proteins into cells. *Proceedings of the National Academy of Sciences of the United States of America* 1994, 91(2):664-668.
25. Zorko M, Langel U: Cell-penetrating peptides: mechanism and kinetics of cargo delivery. *Advanced drug delivery reviews* 2005, 57(4):529-545.
26. Snyder EL, Dowdy SF: Cell penetrating peptides in drug delivery. *Pharmaceutical research* 2004, 21(3):389-393.
27. Richard JP, Melikov K, Vives E, Ramos C, Verbeure B, Gait MJ, Chernomordik LV, Lebleu B: Cell-penetrating peptides. A reevaluation of the mechanism of cellular uptake. *The Journal of biological chemistry* 2003, 278(1):585-590.
28. Jahanshiri F, Eshaghi M, Yusoff K: Identification of phosphoprotein:phosphoprotein and phosphoprotein:nucleocapsid protein interaction domains of the Newcastle disease virus. *Archives of virology* 2005, 150(3):611-618.

29. Romer-Oberdorfer A, Mundt E, Mebatsion T, Buchholz UJ, Mettenleiter TC: Generation of recombinant lentogenic Newcastle disease virus from cDNA. *The Journal of general virology* 1999, 80 (Pt 11):2987-2995.
30. Schwarze SR, Ho A, Vocero-Akbani A, Dowdy SF: In vivo protein transduction: delivery of a biologically active protein into the mouse. *Science* 1999, 285(5433):1569-1572.
31. Yu M, Li X, Huang X, Zhang J, Zhang Y, Wang H: A New Cell Penetrating Peptide (KRP) with Multiple Physicochemical Properties Endows Doxorubicin with Tumor Targeting and Improves Its Therapeutic Index. *ACS applied materials & interfaces* 2018.

Figures

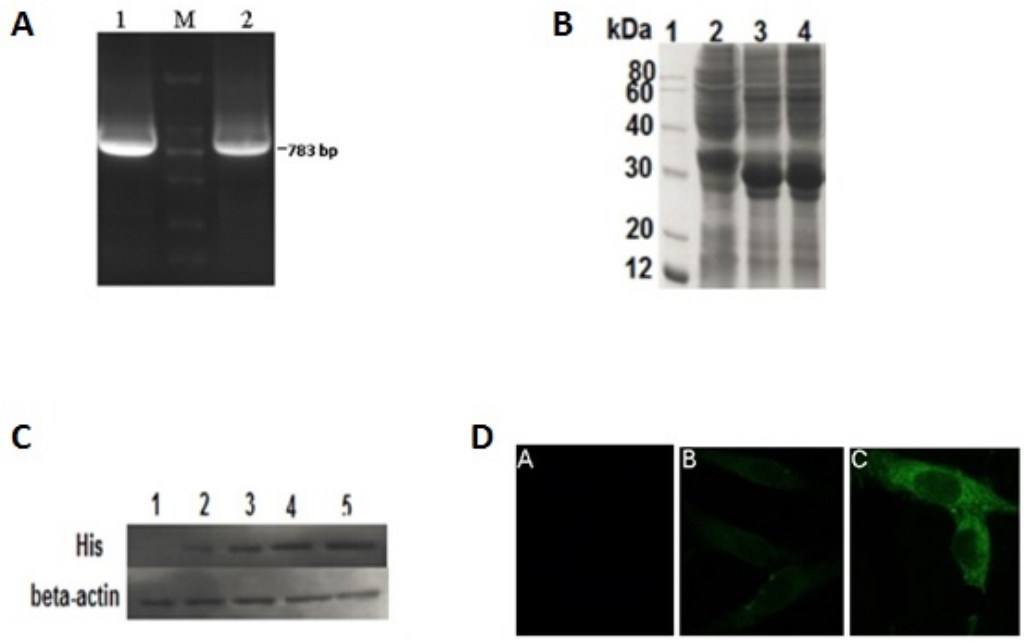

Figure 1

A. Electrophotogram of pET28a-scFv-CTP genes. M. DNA Marker 1.2, pET28a-scFv-CTP ZL.17 and ZL.103 gene; B. Expression of the recombinant plasmid pET28a-scFv-CTP in E.coli. 1. Protein Marker 2. Induced pET28a-scFv-CTP vector control; 3-4. Expression of pET28a-scFv-CTP ZL.17 and ZL.103; C. Western blot analysis of the contration of transduce of CTP-scFv fusion protein. Line 1.negativ control□Line 2. 1μM CTP-scFv□Line 3. 2μM CTP-scFv□ Line 4. 3μM CTP-scFv□Line 5. 4μM CTP-scFv; D.Subcellular localization of CTP-scFv in BHK21 cells.

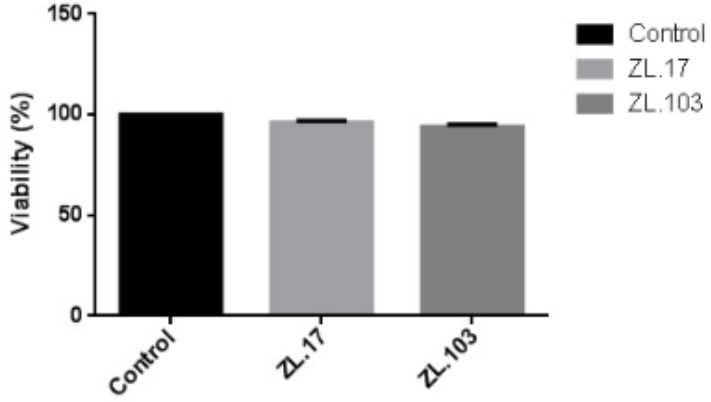

Figure 2

Optical absorbance of the reaction was measured spectrophotometrically at 490 nm with ELISA plate reader. The results shown are the average of three different experiments performed in triplicate.

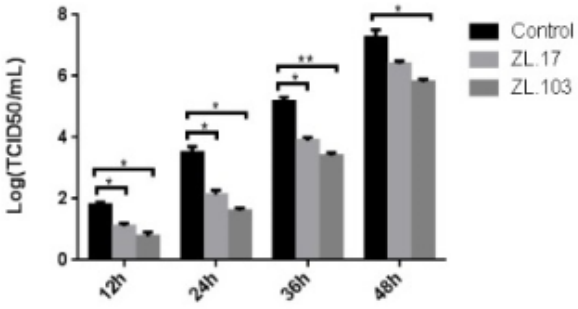

Figure 3

The determination of the effects of anti-P intrabodies on the virus production. BHK21 cell lines expression different intrabodies were infected with Newcatle diseases virus. Virus titer in the culture supernatants was measured at different time points by TCID50 assay. Data shown are from one of the three experiments. **P≤0.01, *P≤0.05

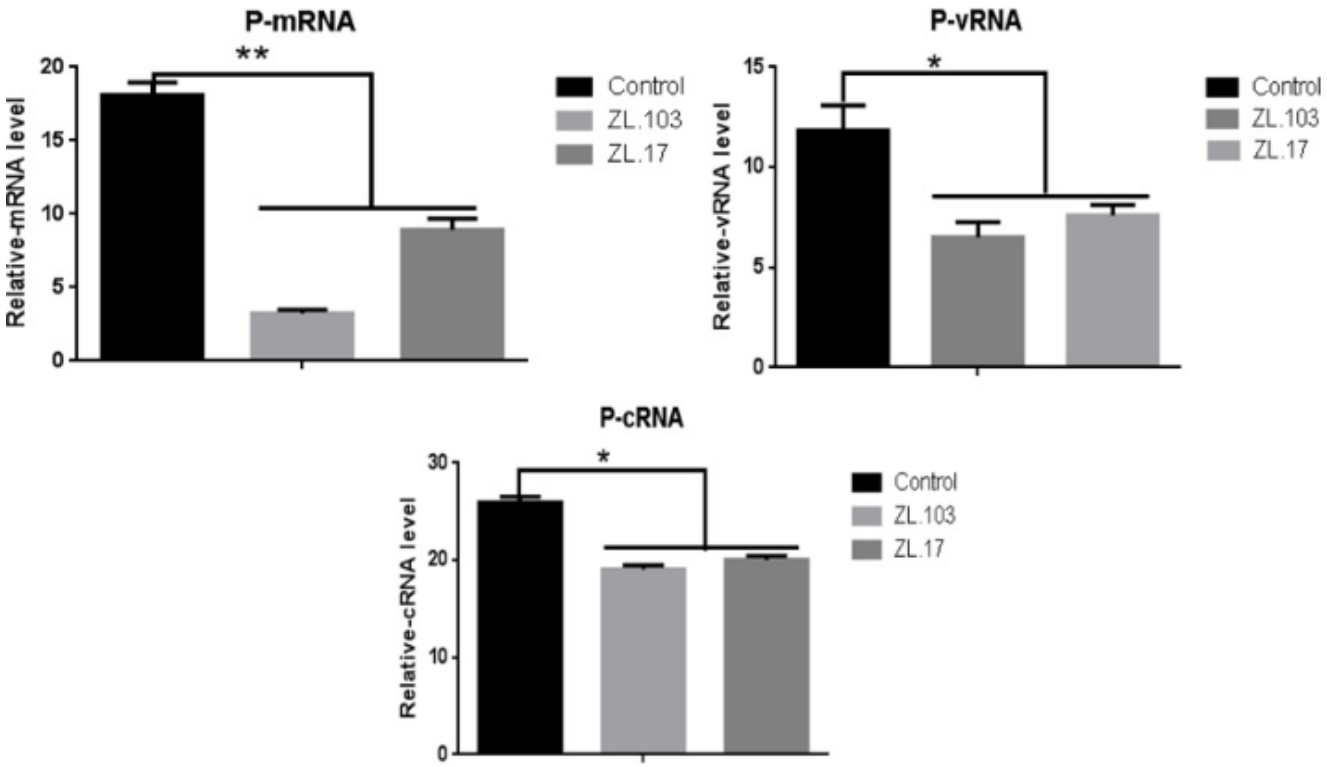

Figure 4

Determination of the role of anti-P intrabodies in Newcastle disease viral RNA production. Data from the real-time PCR showed that the levels of P-specific mRNA were significantly decreased in the presence of ZL.17 and ZL.103. ***P≤0.001, **P≤0.01, *P≤0.05