Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Characterization of Spring Viremia of Carp Virus Glycoprotein Expressed by Recombinant Baculovirus

Fengtao Huang, Qin Li, Xiaoxian Cui[1], Shengbo Cao[1], Chuanxi Xiong, Min Wang, Yuanan Lu[2], Weimin Wang, Jing Ye[1] and Xueqin Liu*

College of Fisheries; [1]College of Veterinary Medicine, Huazhong Agricultural University, Wuhan 430070, China; [2]Department of Public Health Sciences, University of Hawaii at Manoa, USA
*Corresponding author: xueqinliu@mail.hzau.edu.cn

ARTICLE HISTORY

Received: September 27, 2011
Revised: November 25, 2011
Accepted: December 11, 2011

Key words:
Baculovirus
Cell fusion
Expression
Glycoprotein
Insect cell
SVCV

ABSTRACT

Spring viremia of carp (SVC), caused by spring viremia of carp virus (SVCV), is an important disease that causes significant losses in carp fisheries in many countries. The glycoprotein of spring viremia of carp virus (SVCV/G) is the major viral antigen and plays an important role in viral infection. In this study, SVCV/G gene was cloned and inserted into the genome of baculovirus under the control of p10 promoter. SVCV/G was successfully expressed in insect cells. It was found SVCV/G can mediate insect cell fusion and SVCV/G that is similar to the glycoprotein of vesicular stomatitis virus (VSV/G), was detected on the infected insect cell surface by indirect immunofluorescence assay. The size of SVCV/G expressed in insect cells was demonstrated to be approximately 66KDa by Western blot. Comparative analysis of the cell fusions mediated by SVCV/G and VSV/G showed that SVCV/G can cause more severe insect cell fusions. From the data obtained from this study, we speculate that SVCV/G may be incorporated into the recombinant baculovirus envelope, and the recombinant baculovirus harboring SVCV/G on its surface may potentially be used as an effective vaccine against SVCV.

To Cite This Article: F Huang, Q Li, X Cui, S Cao, C Xiong, M Wang, Y Lu, W Wang, J Ye and X Liu, 2012. Characterization of spring viremia of carp virus glycoprotein expressed by recombinant baculovirus. Pak Vet J, 32(3): 334-338.

INTRODUCTION

Spring viremia of carp virus (SVCV) is a member of the genus *Vesiculovirus* of the family *Rhabdoviridae* (Ahne *et al*., 2002; Liu *et al*., 2008). Spring viremia of carp (SVC), which is caused by SVCV and is listed by Office International des Epizooties (OIE) as a notifiable disease, is an acute hemorrhagic and contagious disease in common carp (*Cyprinus carpio*) and several other fish species such as grass carp (*Ctenopharyngodon idella*), crucian carp (*Carassius carassius*), goldfish (*Carassius auratus*), bighead carp (*Aristichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*), tench (*Tinca tinca*) and sheatfish (*Silurus glanis*) (Chen *et al*., 2008; Emmenegger and Kurath, 2008; Liu *et al*., 2008). Outbreaks of SVC can cause significant losses in carp fisheries (Ahne *et al*., 2002; Kanellos *et al*., 2006; Chen *et al*., 2008). The SVCV genome is a linear single-stranded and negative-sense RNA and encodes five structural proteins: nucleoprotein (N protein), phosphoprotein (P protein), matrix protein (M protein), glycoprotein (G protein) and viral RNA-dependent RNA polymerase (L protein) (Ahne *et al*., 2002; Hoffmann *et al*., 2002; Teng *et al*., 2007). SVCV/G exists as trimers on the virus surface that are responsible for attachment to cell membrane receptors and trigger viral endocytosis (Padhi and Verghese, 2008; Zhang *et al*., 2009). SVCV/G is a major antigen which elicits an immune response (Emmenegger and Kurath, 2008). Most monoclonal antibodies against SVCV can react with SVCV/G and DNA vaccines containing the SVCV/G gene can protect fish against SVCV (Reschova *et al*., 2007; Kanellos *et al*., 2006; Emmenegger and Kurath, 2008; Chen *et al*., 2008). Thus, we are interested in studying on SVCV/G due to its role in immune protection against SVCV.

The baculovirus expression vector system has been widely used for production of recombinant proteins since the early 1980s (Hu *et al*., 2008). In 1989, VSV/G was expressed in insect cells successfully, and it was found that VSV/G can mediate insect cell fusion at low pH

(Bailey *et al*., 1989). It was demonstrated that VSV/G was anchored to the infected insect cell surface and was incorporated into the recombinant baculovirus envelope during virus budding (Barsoum *et al*., 1997; Tani *et al*., 2001; Tani *et al*., 2003). Both SVCV and vesicular stomatitis virus (VSV) belong to the genus *Vesiculovirus* of the family *Rhabdoviridae.* SVCV/G is composed of 509 amino acids, and shares some sequence identity (about 32.1%) with VSV/G (Hoffmann *et al*., 2002). To the best of our knowledge, the characterization of SVCV/G in eukaryotic cells is not well addressed. In this study, we describe the characterization of SVCV/G expressed in insect cells by recombinant baculovirus.

MATERIALS AND METHODS

Cell culture and construction of recombinant baculoviruses: Insect Sf9 cells were grown in Grace's insect medium containing 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 μg/ml streptomycin at 27°C.

SVCV/G gene was amplified from the recombinant plasmid pFastBac-SVCV/G which was constructed in our laboratory (data not shown), and VSV/G gene was amplified from the recombinant plasmid pFastBac-VSV/G which was described previously (Wang *et al*., 2007). SVCV/G gene and VSV/G gene were inserted into SmaI-SphI sites of the recombinant plasmid pFastBac-CMV-EGFP, which was also constructed in our laboratory previously (Huang *et al*., 2011), to generate the recombinant plasmids pFastBac-SVCV/G-CMV-EGFP and pFastBac-VSV/G-CMV-EGFP, respectively (Fig. 1). Bac-SVCV/G-CMV-EGFP and Bac-VSV/G-CMV-EGFP were subsequently generated based on the recombinant plasmids pFastBac-SVCV/G-CMV-EGFP and pFastBac-VSV/G-CMV-EGFP using the Bac-to-Bac system (Invitrogen) and following the manufacturer's instructions. Another recombinant baculovirus Bac-CMV-EGFP, which was also constructed in our laboratory previously (Huang *et al*., 2011), was used as the control in this study. All of the above recombinant baculoviruses were propagated in Sf9 cells, and stored at −80°C. Recombinant baculoviruses were tittered by plaque assay.

Immunofluorescence and confocal microscopy analysis: Sf9 cells were seeded into cell culture dishes (NEST Biotechnology Co.LTD.) and infected with Bac-SVCV/G-CMV-EGFP or Bac-CMV-EGFP. At 45 h post-infection, the cells were fixed with absolute methanol and blocked with 5% normal goat serum and 1% BSA in PBS. Rabbit polyclonal antibodies against SVCV (1: 500; kindly provided by professor Lu Yuanan, University of Hawaii, USA) and Alexa Fluor® 555-conjugated goat anti-rabbit IgG (1:400; Invitrogen) were used as primary antibody and secondary antibody, respectively. Localization of SVCV/G was visualized using a confocal microscope (LSM 510 Meta, Carl Zeiss).

Western blot analysis: Infected cells were lysed in SDS sample buffer and SDS-PAGE was performed. Separated proteins were transferred to a nitrocellulose membrane. SVCV/G expressed in insect cells was detected using Rabbit polyclonal antibodies against SVCV (1: 500), followed by goat anti-rabbit IgG (1: 1000). Protein band on the membrane was visualized by BeyoECL Plus reagent (Beyotime, China).

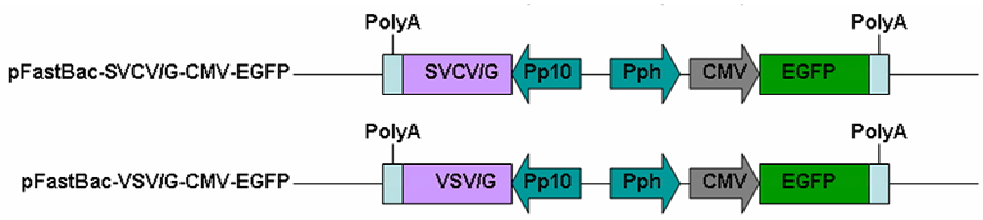

Fig. 1: Schematic diagram of the recombinant plasmids for construction of recombinant baculoviruses. The structure of the plasmids based on pFastBac™Dual is displayed. Pp10, p10 promoter; Pph, polyhedrin promoter; CMV, cytomegalovirus immediate-early promoter; SVCV/G, glycoprotein of spring viremia of carp virus; VSV/G, glycoprotein of vesicular stomatitis virus; EGFP, enhanced green fluorescent protein; PolyA, polyadenylation signal.

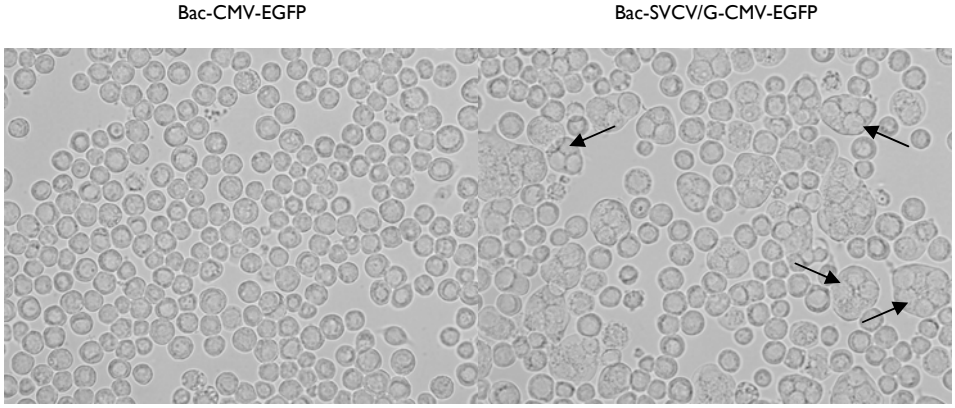

Fig. 2: Morphology of infected Sf9 cells. Sf9 cells were infected with Bac-SVCV/G-CMV-EGFP or Bac-CMV-EGFP at an MOI of 0.1 and were observed under an inverted microscope at 35 h post-infection. Arrows denote the cell fusions in Bac-SVCV/G-CMV-EGFP-infected Sf9 cells.

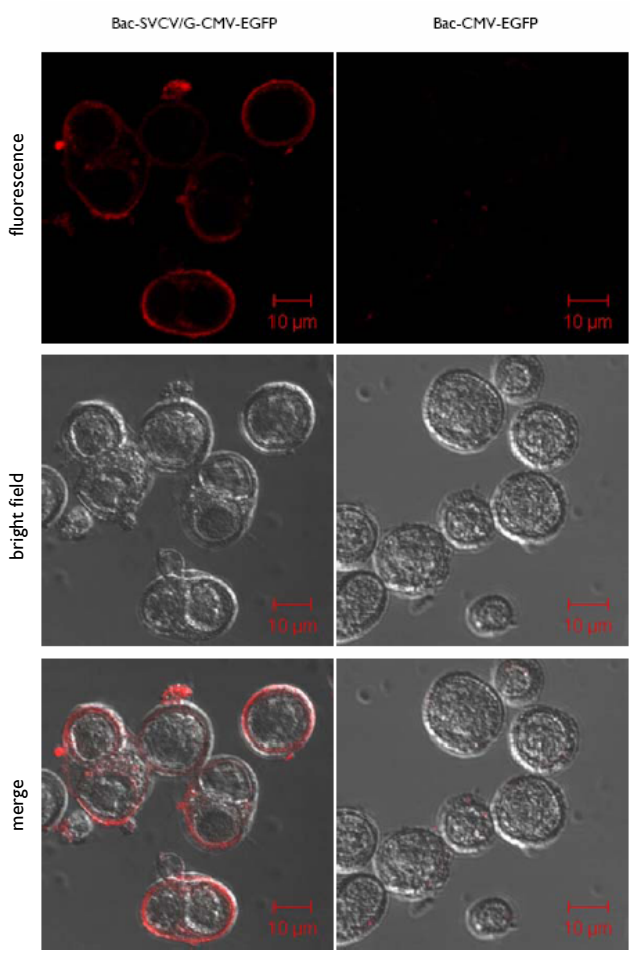

Fig. 3: Localization of SVCV/G on the plasma membrane of infected Sf9 cells. Cells were infected with Bac-CMV-EGFP or Bac-SVCV/G-CMV-EGFP at an MOI of 0.5. At 45 h post-infection, the expression of SVCV/G was detected by indirect immunofluorescence and the localization of SVCV/G was visualized using a confocal microscope.

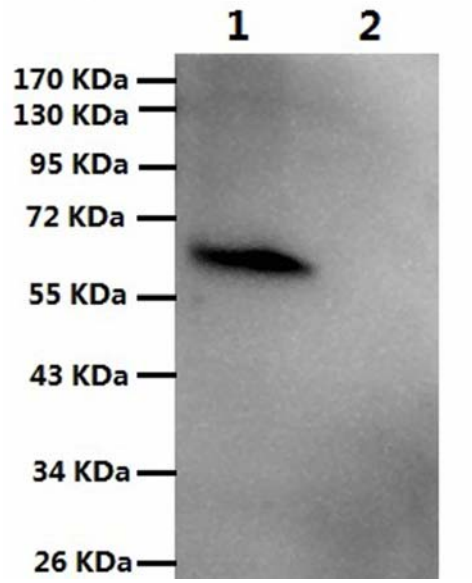

Fig. 4: Western blot analysis of SVCV/G expressed in insect cells. Cells were infected with Bac-CMV-EGFP or Bac-SVCV/G-CMV-EGFP at an MOI of 0.2 and collected at 36 h post-infection. Lane 1, Insect cells infected with Bac-SVCV/G-CMV-EGFP; Lane 2, Insect cells infected with Bac-CMV-EGFP.

RESULTS

Cell fusion induced by SVCV/G: To express SVCV/G and VSV/G in insect cells, two recombinant baculoviruses Bac-SVCV/G-CMV-EGFP and Bac-VSV/G-CMV-EGFP were constructed. SVCV/G gene and VSV/G gene were successfully amplified and inserted into recombinant plasmid pFastBac-CMV-EGFP, respectively. The recombinant baculoviruses Bac-SVCV/G-CMV-EGFP and Bac-VSV/G-CMV-EGFP were obtained according to the manufacturer's instructions.

In a previous study, it was shown that VSV/G can induce cell fusion in insect cells (Bailey *et al*., 1989). In this study, cell fusion was also caused by SVCV/G. The Sf9 cells were infected with Bac-CMV-EGFP or Bac-SVCV/G-CMV-EGFP at an MOI of 0.1 and were observed under an inverted microscope (Olympus IX70) at 35 h post-infection. As is shown in figure 2, cell fusions can be caused in Bac-SVCV/G-CMV-EGFP-infected cells. However, there is no cell fusion found in Bac-CMV-EGFP-infected cells, indicating SVCV/G also has a fusion activity in insect cells.

Detection of SVCV/G expressed in insect cells by indirect immunofluorescence and Western blot: SVCV/G demonstrated fusion activity in insect cells. Therefore, we speculated that SVCV/G may be expressed on the insect cell surface. To investigate the cellular localization of SVCV/G, insect cells were infected with Bac-CMV-EGFP or Bac-SVCV/G-CMV-EGFP at an MOI of 0.5. At 45 h post-infection, the expression of SVCV/G was detected by indirect immunofluorescence and the localization of SVCV/G was visualized using a confocal microscope. As expected, SVCV/G was located on the infected insect cell surface (Fig. 3).

Western blot analysis was utilized to further characterize expression of SVCV/G in insect cells. Insect cells were infected with Bac-CMV-EGFP or Bac-SVCV/G-CMV-EGFP at an MOI of 0.2. At 36 h post-infection, cells were collected and analyzed by Western blot. Western blot analysis showed that an approximately 66 KDa band was present in the Bac-SVCV/G-CMV-EGFP-infected cells and not present in the Bac-CMV-EGFP-infected cells (Fig. 4).

Comparison of cell fusions mediated by SVCV/G and VSV/G in insect cells: In previous studies, it was found that VSV/G is expressed on the insect cell surface and can mediate cell fusion (Bailey *et al*., 1989). In the present study, we found SVCV/G can also mediate cell fusion and is located on the infected insect cell surface. For comparison of cell fusions mediated by SVCV/G and VSV/G, insect cells were infected with Bac-SVCV/G-CMV-EGFP, Bac-VSV/G-CMV-EGFP or Bac-CMV-EGFP at an MOI of 0.5. At 24 h and 48 h post-infection, the infected cells were observed under an inverted microscope. Numerous cell fusions occurred in Bac-SVCV/G-CMV-EGFP-infected insect cells as early as 24 h post-infection, and cell fusions became more severe at 48 h post-infection. However, in Bac-VSV/G-CMV-EGFP-infected insect cells, cell fusions were scarce at 24 h post-infection and less severe than that in Bac-SVCV/G-CMV-EGFP-infected insect cells at 48 h post-infection

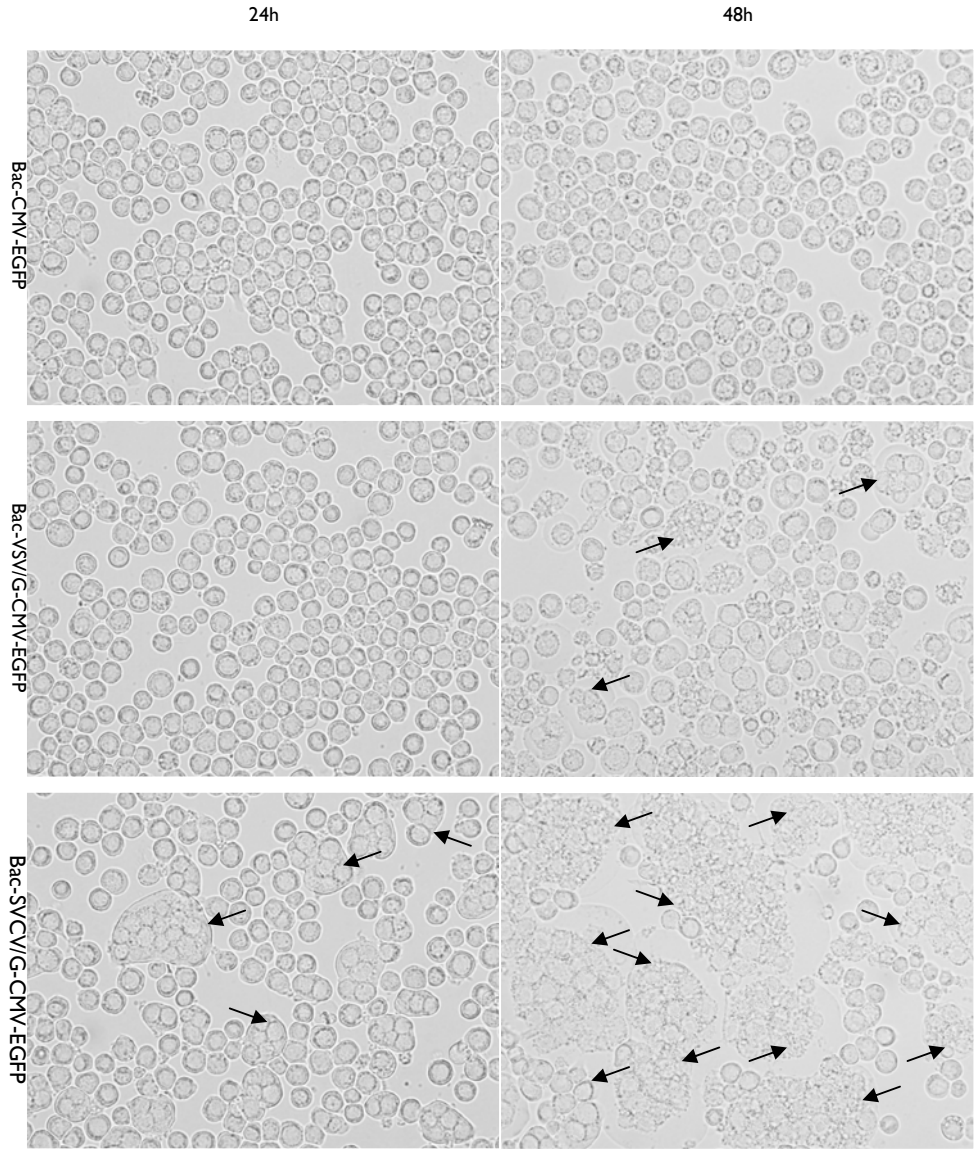

Fig. 5: Comparison of cell fusions in baculovirus-infected Sf9 cells. Cells were infected with Bac-CMV-EGFP, Bac-VSV/G-CMV-EGFP or Bac-SVCV/G-CMV-EGFP at an MOI of 0.5. Cell fusions were observed under an inverted microscope at 24 h and 48 h post-infection. Arrows indicate cell fusions.

No cell fusion was observed in Bac-CMV-EGFP-infected insect cells. Through comparison of the cell fusions mediated by SVCV/G and VSV/G, it was found that SVCV/G can cause more severe insect cell fusions (Fig. 5).

DISCUSSION

In the present study, SVCV/G was successfully expressed by recombinant baculovirus. It was found that SVCV/G can induce cell fusion in insect cells. Subsequently, it was demonstrated that SVCV/G was located on the infected insect cell surface. Similarly, in other rhabdoviruses, such as VSV, rabies virus, viral hemorrhagic septicemia virus (VHSV), bovine ephemeral fever virus (BEFV), the G proteins expressed in insect cells were located on the insect cell surface and showed a fusion activity (Bailey *et al*., 1989; Tuchiya *et al*., 1992; Lecocq-Xhonneux *et al*., 1994; Johal *et al*., 2008). Interestingly, although the rhabdoviruses differ, their G proteins can induce cell fusion and are expressed on the infected insect cell surface. In previous studies, it was found that VSV/G expressed on the insect cell surface can rescue virion propagation and infectivity of GP64-null baculovirus (Mangor *et al*., 2001; Zhou and Blissard, 2008). It will be of interest to determine whether SVCV/G can rescue infectivity of GP64-null baculovirus. Additionally, we found SVCV/G expressed in insect cells had a molecular mass of approximately 66 KDa, which was larger than the calculated molecular mass of SVCV/G (about 57.4 KDa) (Hoffmann *et al*., 2002). However, it was slightly smaller than the native viral protein (about 69 KDa) (Chen *et al*., 2008). In previous studies, G proteins of other rhabdoviruses, such as rabies virus, VHSV, BEFV, have also been found slightly smaller than the native viral protein (Tuchiya *et al*., 1992; Lecocq-Xhonneux *et al*., 1994; Johal *et al*., 2008). This may be due to different glycosylation levels between insect cells

and vertebrate cells. Furthermore, compared with cell fusions mediated by VSV/G, we also found SVCV/G can mediate more severe insect cell fusion. It has been shown that cell fusion mediated by VSV/G was low-pH-dependent and was inhibited if the pH was above 6.2 (Bailey *et al*., 1989). It seems SVCV/G shows a fusion activity at a slightly higher pH than VSV/G.

Recombinant baculovirus harboring antigen protein displayed on its envelope can elicit specific immune response and has been proven as an effective immunogen (Xu *et al*., 2008; Musthaq *et al*., 2009; Xu *et al*., 2009; Meng *et al*., 2011; Xu *et al*., 2011). In previous studies, it was demonstrated that VSV/G can be translocated to the insect cell surface and incorporated into the baculovirus envelope during virus budding (Barsoum *et al*., 1997; Tani *et al*., 2001; Tani *et al*., 2003). Thus, the recombinant baculovirus harboring VSV/G may be used as an effective vaccine like other antigen proteins displayed on the baculovirus envelope. As SVCV/G shares some sequence identity with VSV/G (Hoffmann *et al*., 2002) and is located on the surface of insect cells, we speculate SVCV/G may also be incorporated into the recombinant baculovirus envelope and the recombinant baculovirus Bac-SVCV/G-CMV-EGFP may potentially be used as an effective vaccine against SVCV.

In conclusion, it was demonstrated that SVCV/G was located on the infected insect cell surface and may induce insect cell fusion, which is similar to VSV/G. Furthermore, SVCV/G can cause more severe cell fusions than VSV/G in insect cells. It was the first time to characterize SVCV/G expressed in insect cells. The findings may be useful for development of an effective vaccine against SVCV in future.

Acknowledgements: This research work was supported by Natural Science Foundation of China (30901118, 31172433), 973 Project of China (No. 2010CB530100), Fundamental Research Funds for the Central Universities (2011PY121) and Natural Science Foundation of Hubei Province (2008CBB099).

REFERENCES

Ahne W, HV Bjorklund, S Essbauer, N Fijan, G Kurath and JR Winton, 2002. Spring viremia of carp (SVC). Dis Aquat Organ, 52: 261-272.

Bailey MJ, DA McLeod, CY Kang and DH Bishop, 1989. Glycosylation is not required for the fusion activity of the G protein of vesicular stomatitis virus in insect cells. Virology, 169: 323-331.

Barsoum J, R Brown, M McKee and FM Boyce, 1997. Efficient transduction of mammalian cells by a recombinant baculovirus having the vesicular stomatitis virus G glycoprotein. Hum Gene Ther, 8: 2011-2018.

Chen ZY, H Liu, ZQ Li and QY Zhang, 2008. Development and characterization of monoclonal antibodies to spring viremia of carp virus. Vet Immunol Immunopathol, 123: 266-276.

Emmenegger EJ and G Kurath, 2008. DNA vaccine protects ornamental koi (Cyprinus carpio koi) against North American spring viremia of carp virus. Vaccine, 26: 6415-6421.

Hoffmann B, H Schutze and TC Mettenleiter, 2002. Determination of the complete genomic sequence and analysis of the gene products of the virus of Spring Viremia of Carp, a fish rhabdovirus. Virus Res, 84: 89-100.

Hu YC, K Yao and TY Wu, 2008. Baculovirus as an expression and/or delivery vehicle for vaccine antigens. Expert Rev Vaccines, 7: 363-371.

Huang F, S Cao, X Cui, C Xiong, M Wang, Y Lu, W Wang, J Ye and X Liu, 2011. Efficient gene delivery into fish cells by an improved recombinant baculovirus. J Virol Methods, 173: 294-299.

Johal J, K Gresty, K Kongsuwan and PJ Walker, 2008. Antigenic characterization of bovine ephemeral fever rhabdovirus G and GNS glycoproteins expressed from recombinant baculoviruses. Arch Virol, 153: 1657-1665.

Kanellos T, ID Sylvester, F D'Mello, CR Howard, A Mackie, PF Dixon, KC Chang, A Ramstad, PJ Midtlyng and PH Russell, 2006. DNA vaccination can protect Cyprinus Carpio against spring viremia of carp virus. Vaccine, 24: 4927-4933.

Lecocq-Xhonneux F, M Thiry, I Dheur, M Rossius, N Vanderheijden, J Martial and P de Kinkelin, 1994. A recombinant viral haemorrhagic septicaemia virus glycoprotein expressed in insect cells induces protective immunity in rainbow trout. J Gen Virol, 75: 1579-1587.

Liu Z, Y Teng, X Xie, H Li, J Lv, L Gao, F Tian, Y Jiang, Z Chu, C Xie and H Liu, 2008. Development and evaluation of a one-step loop-mediated isothermal amplification for detection of spring viremia of carp virus. J Appl Microbiol, 105: 1220-1226.

Mangor JT, SA Monsma, MC Johnson and GW Blissard, 2001. A GP64-null baculovirus pseudotyped with vesicular stomatitis virus G protein. J Virol, 75: 2544-2556.

Meng T, AB Kolpe, TK Kiener, VT Chow and J Kwang, 2011. Display of VP1 on the surface of baculovirus and its immunogenicity against heterologous human enterovirus 71 strains in mice. PLoS One, 6: e21757.

Musthaq S, S Madhan, AS Sahul Hameed and J Kwang, 2009. Localization of VP28 on the baculovirus envelope and its immunogenicity against white spot syndrome virus in Penaeus monodon. Virology, 391: 315-324.

Padhi A and B Verghese, 2008. Detecting positively selected codons in the glycoprotein of spring viremia of carp virus (SVCV) isolates from the USA and China. J Fish Dis, 31: 785-791.

Reschova S, D Pokorova, Z Nevorankova, J Hulova and T Vesely, 2007. Detection of spring viremia of carp virus (SVCV) with monoclonal antibodies. Vet Med, 52: 308-316.

Tani H, CK Limn, CC Yap, M Onishi, M Nozaki, Y Nishimune, N Okahashi, Y Kitagawa, R Watanabe, R Mochizuki, K Moriishi and Y Matsuura, 2003. In vitro and in vivo gene delivery by recombinant baculoviruses. J Virol, 77: 9799-9808.

Tani H, M Nishijima, H Ushijima, T Miyamura and Y Matsuura, 2001. Characterization of cell-surface determinants important for baculovirus infection. Virology, 279: 343-353.

Teng Y, H Liu, JQ Lv, WH Fan, QY Zhang and QW Qin, 2007. Characterization of complete genome sequence of the spring viremia of carp virus isolated from common carp (Cyprinus carpio) in China. Arch Virol, 152: 1457-1465.

Tuchiya K, Y Matsuura, A Kawai, A Ishihama and S Ueda, 1992. Characterization of rabies virus glycoprotein expressed by recombinant baculovirus. Virus Res, 25: 1-13.

Wang S, L Fang, H Fan, Y Jiang, Y Pan, R Luo, Q Zhao, H Chen and S Xiao, 2007. Construction and immunogenicity of pseudotype baculovirus expressing GP5 and M protein of porcine reproductive and respiratory syndrome virus. Vaccine, 25: 8220-8227.

Xu XG, DW Tong, MT Chiou, YC Hsieh, WL Shih, CD Chang, MH Liao, YM Zhang and HJ Liu, 2009. Baculovirus surface display of NS3 nonstructural protein of classical swine fever virus. J Virol Methods, 159: 259-264.

Xu XG, MT Chiou, YM Zhang, DW Tong, JH Hu, MT Zhang and HJ Liu, 2008. Baculovirus surface display of E(rns) envelope glycoprotein of classical swine fever virus. J Virol methods, 153: 149-155.

Xu XG, ZS Wang, Q Zhang, ZC Li, HN Zhao, W Li, DW Tong and HJ Liu, 2011. Baculovirus surface display of E envelope glycoprotein of Japanese encephalitis virus and its immunogenicity of the displayed proteins in mouse and swine models. Vaccine, 29: 636-643.

Zhang NZ, LF Zhang, YN Jiang, T Zhang and C Xia, 2009. Molecular analysis of spring viremia of carp virus in China: a fatal aquatic viral disease that might spread in East Asian. PLoS One, 4: e6337.

Zhou J and GW Blissard, 2008. Display of heterologous proteins on gp64null baculovirus virions and enhanced budding mediated by a vesicular stomatitis virus G-stem construct. J Virol, 82: 1368-1377.