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Identification and Characterization of the CGSIV Protein Interactors of the Host Cyprini Ubc-9

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Abstract

Chinese giant salamander Iridovirus is a virus with high lethality and infectivity. The research of CGSIV molecular biology can enhance our understanding of the possible function in viral infection and mechanisms. Research shows that a number of viral proteins known to related with the SUMO pathway at many levels, like viral replication, assembly and immune evasion. Our study was attempt to investigate the interplay between the host SUMO pathway and CGSIV viral protein. In this paper, we used Ubc-9 as a bait protein to screen CGSIV DNA library by yeast two-hybrid system. Then verified the interaction of CGSIV protein and Ubc-9 by GST Pull-down and BiFC. Experiments showed some viral proteins can interact with Ubc-9 protein, which may be modified by SUMOylation system. We used Yeast two hybrid screened out seven CGSIV viral proteins that may interact with Ubc-9: two largest subunits of DNA-dependent RNA polymerase (CGSIV 9R and 49R ORF), ICP-46 protein (CGSIV 18L ORF), homology of TFIIS factor (CGSIV 30L ORF), DNA polymerase (CGSIV 50L ORF), 37R and 57L. CGSIV 57L is the only known protein with SAP domain in CGSIV that may be essential in transcription. GST Pull-down and BiFC further confirmed the interaction between CGSIV 57L and Ubc-9, suggesting that CGSIV 57L might be modified by SUMOylation system. Provide a clue for exploring the relationship between viral protein and host SUMO system.

Keywords

Chinese Giant Salamander Iridovirus; Protein Interaction; Ubc-9; CGSIV 57L ORF.

1. Introduction

Chinese giant salamander iridovirus (CGSIV) is a virus isolated from the Chinese giant salamander (Andrias davidianus) [1,2], belongs to the genus Ranavirus (family Iridoviridae). Iridoviruses are double-stranded DNA viruses [3,4], which are widely distributed in nature and can infect a variety of invertebrates and vertebrates [5,6], such as turtles, frogs, fish, shrimp and beetles [7]. At present, the family Iridoviridae contains six different genera [8]: Lymphocystivirus, Ranavirus, Megalocytivirus, Iridovrius, Chloriridovrius, and Decapodiridovirus.

Iridoviridae and Ranavirus are the main research areas in viral etiology, molecular biology and disease control. Small ubiquitin-like modifier (SUMO)ylation is a post-translational modification mechanism that regulate a large number of cellular processes and control the functions of target proteins [9]. Previous studies have shown that many viral proteins are known to be modified by SUMOylation system to exsert their function [10], like affect their viral replication [10]. Studies have shown that over-expressed two SUMO homolog genes (EcSUMO1 and EcSUMO2) from Epinephelus coioides enhanced Singapore grouper iridovirus (SGIV) and Red-spotted grouper nervous necrosis virus (RGNNV) replication [11]. Our research has previously proved that the over-expressed or knock-down of ubiquitin conjugating 9 (Ubc-9) could lead to the increase or decrease of CGSIV replication. It can be speculated that the virus might affect their own replication using the SUMO system. How does the virus use the SUMO system to affect their own replication? E2 enzyme

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Ubc-9 is the only known E2 enzyme in SUMO system [12], as the core protein of SUMO system is probably to interact with viral proteins and regulate viral replication by SUMOylation.

The aim of this paper is focus to investigate whether CGSIV encoded proteins as target proteins of SUMOylation by interacting with Ubc-9. We screened the interplay between viral proteins and the Ubc-9 from the host cells Epithelioma Papilloma Cyprini (EPC) by Yeast two-hybrid system (Y2H), Glutathione S transferase Pull-down (GST Pull-down) and bimolecular fluorescence complementation (BiFC) assays, so as to provide more information for studying the function of virus protein SUMO and the interaction between host SUMO system and virus life cycle.

2. Materials and Methods

2.1 Virus, cells, strains and plasmids

EPC cells was cultured at 26℃ in medium 199 (M199), medium contained 10% fetal bovine serum (FBS) and 100 U/mL penicillin and 100 μg/mL streptomycin. CGSIV was incubated with EPC cells; Escherichia coli DH5α, Rosetta DE3, Prokaryotic expression vectors pET22b and pGEX6P-1, Plasmids pcDNA3.1(+)-NmCherry, pcDNA3.1(+)-CmCherry, pGADT7-HC, pcDNA3.1-EGFP-NmCherry, pcDNA3.1-CmCherry-EGFP and pcDNA3.1-Ubc-9 were previously prepared by our laboratory.

2.2 Main materials

Medium 199, Opit-MEM reduced serum Medium, FBS and phosphate buffer saline (PBS) were purchased from Gibco. Viral DNA kit, Plasmid mini kit and cycle pure kit were purchased from OMEGA. Restriction endonuclease, T4 ligase, Ex taq DNA polymerase, DNA Blunting Kit, DNA A-Tailing Kit and pMD18-T Vector Cloning Kit were purchased from TAKARA. GST-tag antibodies, HIS-tag antibody, goat anti-mouse secondary antibody and goat anti-rabbit secondary antibody were purchased from Proteintech. GST-Sefinose Resin was purchased from Novagen Company. Bacterial lysis solution (ultrasound free) was purchased from BestBio. PolyHigh non-liposome efficient transfection reagent was purchased from Sangon Biotech. Isopropyl-b-d-thiogalactopyranoside (IPTG) was purchased from sigma. Using Clontech yeast two-hybrid system (including Saccharomyces cerevisiae Y187 and Y2H Gold, vector pGADT7, pGBKT7, pGADT7-T, pGBKT7-53, pGBKT7-LAM). PCR primers were synthesized by Beijing Genomics institution.

2.3 Design of PCR primers

CGSIV Genome were downloaded from NCBI nucleotide database. Primers were designed by Primer premier software. The underline is the restriction endonuclease site.

Table 1. List of primer pairs used for PCR

Primer name	Primer sequence (5'→3')
M13 RV-M	GAGCGGATAACAATTTCACACAGG
M13-47	CGCCAGGGTTTTCCCAGTCACGAC
EPC-Ubc-9-FP	ATAAACATATGTCTGGCATTGCTCTG
EPC-Ubc-9-RP	TATTTGAATTCTTACGACGGGGAG
SMARTIII-18T-FP	AAGCAGTGGTATCAACGCAGAGTGGCCATTATGGCCGGGGAGCGGATAACAATTTCACACAGG
CDSIII-18T-RP	ATTCTAGAGGCCGAGGCGGCCGACATGCGCCAGGGTTTTCCCAGTCACGAC
5smart-FP	TTCCACCCAAGCAGTGGTATCAACGCAGAGTGG
3CDS-RP	GTATCGATGCCCACCCTCTAGAGGCCGAGGCGGCCGACA
y-AD-FP	CGATGATGAAGATACCC
y-AD-RP	AGAAATTGAGATGGTGC
AD-57L-FP-E	ATAAAAGAATTCATGCAAGTCTACTCTCC
AD-57L-RP-X	ATAAAACTCGAGCTAACACAGATAATCTTCAGT
pET22b-Ubc-9-FP	ATAAAAAAGCTTATGGAAATGTTTGCATCTAAATCTC
pET22b-Ubc-9-RP	ATAAAGAATTCTCGCCACTCAAAGGAT
pGEX6P-1-57L-FP	ATAAAAGAATTCATGCAAGTCTACTCTCCTTC
pGEX6P-1-57L-RP	ATAAAAGCGGCCGCCTAACACACATAATCTTCA
3.1-Ubc-9-Cm-FP-E	ATAAAGAATTCCATGTCTGGCATTGCTCTG
3.1-Ubc-9-Cm-RP-H	ATAAAGAATTCTCGCCACTCAAAGGAT
3.1-57L-Nm-FP-E	ATAAAAGAATTCATGCAAGTCTACTCTCCTTC
3.1-57L-Nm-RP-N	ATAAAAGCGGCCGCACACAGATAATCTTCAGTCCA

ISSN: 2414-1895 DOI: 10.6919/ICJE.202103_7(3).0037

2.4 Construction DNA genomic library of CGSIV

To propagate the CGSIV virus, the EPC cells were infected and incubated at 26℃. Collected the solution of CGSIV after infected 48 to 72 hours. Using Viral DNA kit to extracte CGSIV genome DNA. The CGSIV genomic DNA was broken with the ultrasonic cell disrupter and it was randomly broken into different sizes of DNA fragments. Then analyzed using agarose gel electrophoresis. The fragment of viral DNA was purified using the Cycle Pure Kit and blunting by a DNA Blunting Kit, added with A tail, then ligated with pMD18-T vector. Transformed plasmid into E. coli DH5α to obtain the recombinant genomic library plasmid pMD18-T-CGSIV DNA. Since the sequence of pMD18-T vector is known, regard the pMD18-T-CGSIV genomic library plasmid as a template, using universal primers of 18-T vector M13 RV-M and M13-47 (Table 1) to analyze the inserted virus DNA fragment by PCR.

2.5 Yeast two-hybrid library screening using yeast mating

2.5.1 Generate bait plasmid pGBKT7-Ubc-9

Using pcDNA3.1-Ubc-9 as template, the Ubc-9 gene was amplified by PCR using primers EPC-Ubc-9-FP and EPC-Ubc-9-RP (Table 1) and cloned into pGBKT7 to generate the bait plasmid pGBKT7-Ubc-9. Transformed plasmid into E. coli DH5α. Then selected the positive clones from ampicillin resistance plate. After PCR identification, sent the positive clones for DNA sequencing.

2.5.2 Preparation of CGSIV virus fragment with homologous arms

The viral DNA fragments were ligated to the homologous arms by PCR. Firstly, the pMD18-T-CGSIV genomic library plasmid was used as the template to amplify the target fragment by primers SMARTIII-18T-F and CDSIII-18T-R (Table 1). Using the first PCR product as template, 5smart-FP and 3CDS-RP as primers (Table 1), the complete homologous arms of CGSIV DNA fragments were obtained by PCR. The vector pGADT7-HC has the same homologous arm with CGSIV DNA fragments.

2.5.3 Yeast two-hybrid library screening using yeast mating

Yeast transformed by PEG/LiAC method. Firstly, digest pGADT7-HC vector with Sma-I. Secondly, linear pGADT7-HC and PCR products of viral DNA fragments with homologous arms were co-transformed into yeast Y187, pGBKT7-Ubc-9 was transformed into yeast Y2H Gold. The yeast two-hybrid screen was performed according to the instructions of Clontech. Y187 (pGADT7-CGSIV-DNA) and Y2H Gold (pGBKT7-Ubc-9) were mating and incubated at 28℃ for 24h. The mating culture was plated on SD/-Trp/-Leu/-Ade for 3 to 7 days. Finally, colonies that grew on SD/-Trp/-Leu/-Ade/-His plate were seleted to identify the candidate protein.

2.5.4 Viral fragments identified by PCR

Selected the screened yeast, added 10 μL yeast protein lysate, incubated at 80°C for 20 minutes. The target fragment was amplified by primers y-AD-FP and y-AD-RP (table 1), the lysed yeast as the template. Then analyzed using 1% agarose gel electrophoresis. The PCR products were sent to gene sequencing.

2.6 Yeast two-hybrid assay

Seven CGSIV protein fragments were obtained through yeast two-hybrid screen, one of them was encoded by CGSIV 57L. In order to investigate the relationship between CGSIV 57L protein and EPC Ubc-9, the full-length sequence of CGSIV 57L was cloned into the vector pGADT7. The yeast two-hybrid assay was used to confirm whether EPC Ubc-9 could interact with CGSIV 57L protein. The recombinant plasmid pGADT7-57L was transformed into yeast Y187, combine the Y187 with the bait strain Y2H Gold (pGBKT7-Ubc-9). Screened the positive clones according to the instructions of Clontech.

2.7 GST Pull-down assay

The plasmids pGEX6p-1-57L and pET22b-Ubc-9 were constructed using the indicated primers (Table 1). Protein was expressed by E.coli Rosetta DE3 harboring pGEX6p-1-57L and pET22b-Ubc-

ISSN: 2414-1895 DOI: 10.6919/ICJE.202103_7(3).0037

9 in 0.5 mmol/L IPTG at 18°C for 18 hours. Bacterial samples were lysed according to the instructions of the bacterial lysis solution (ultrasound free). Supernatant of pET22b-Ubc-9 lysis solution was added to the supernatant of pGEX6P-1-57L lysis solution, and added 100 ul 50% glutathione agarose beads, 57L-GST and Ubc-9-his were incubated at 4°C for 18 hours. Washed GST agarose beads repeatedly for 8 to 9 times with bacterial lysis solution. 100°C incubation for 10 minutes. The eluted proteins were analyzed via 12% polyacrylamide gel electrophoresis (PAGE).

2.8 BiFC identified the interaction between CGSIV 57L and EPC Ubc-9 protein

The plasmids pcDNA3.1(+)-57L-Nmcherry and pcDNA3.1(+)-Cmcherry-Ubc-9 were constructed using the indicated primers (Table 1).

Inoculate appropriate amount of EPC cells in six-well plates, before transfection the cell density reached about 80% in 18-24 hours as the ideal state. Wash cells 2 times with PBS, replace the old medium with fresh medium. Prepare transfection reagent/DNA solution: 4μg plasmid DNA dissolved in 200 μL Opit-MEM medium, mix gently, 12 μL PolyHigh transfection reagent dissolved in 200 μL OPit-MEM medium, mix gently, allow to stand at room temperature for 5min, Add PolyHigh transfection reagent diluent to the DNA-containing diluent, mix gently and allow at room temperature for 15minutes. The transfection reagent/DNA solution was added into the corresponding six-well plate cell culture medium, gently mixed and placed in 26℃, 5% CO2 incubator for culture. Replace the fresh medium after 6 to 8 hours. Observation cells after transfected 24 to 48 hours by fluorescence microscope.

3. Results

3.1 Construction DNA genomic library of CGSIV

The prepared CGSIV genomic DNA was analyzed using agarose gel electrophoresis (Figure 1A). The size of CGSIV genomic DNA is about 15000 bp.

The CGSIV genomic DNA was broken with the ultrasonic cell disrupter and it was randomly broken into different sizes of DNA fragments (Figure 1B). 1% agarose gel electrophoresis analyzed. The genomic DNA of CGSIV was broken into 250bp-2500bp under 60W ultrasonic wave.

After the fragment of viral DNA blunting by a DNA Blunting Kit, added with A tail, then ligated into pMD18-T vector to construct the recombinant plasmid pMD18-T-CGSIV genomic library. The inserted viral DNA fragments were identified by PCR (Figure 1C). The inserted DNA fragments in pMD18-T-CGSIV recombinant plasmid were concentrated between 0.25 and 2.5 Kb. It indicated that the CGSIV genomic DNA fragments were successfully inserted into pMD18-T vector.

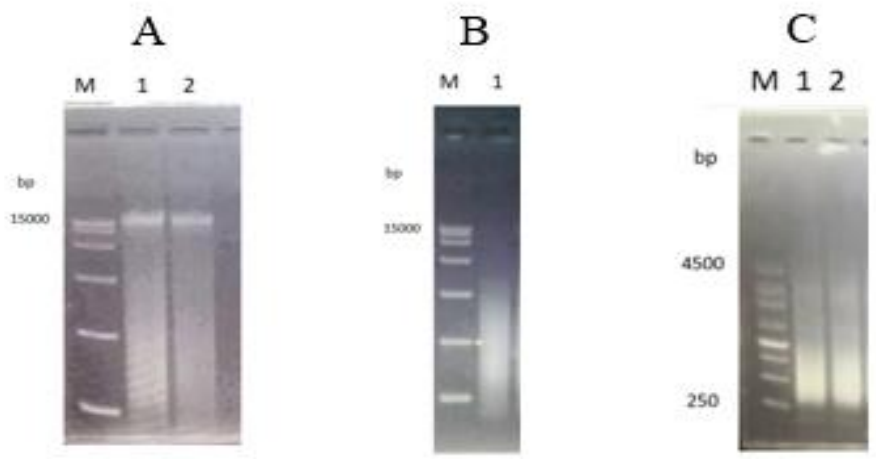

Figure 1. pMD18-T-CGSIV genome DNA library. A:Extraction of CGSIV genome DNA; B:CGSIV genome DNA broken by ultrasomic; C:CGSIV genome DNA library by PCR

ISSN: 2414-1895 DOI: 10.6919/ICJE.202103_7(3).0037

3.2 Yeast two-hybrid screened CGSIV protein using yeast mating

The yeasts screened by yeast two-hybrid system were lysed and identified by PCR. The results of 1% agarose gel electrophoresis are shown in Figure 2. The PCR results showed that there were multiple fragments of different sizes, indicating that different protein fragments that might interact with EPC Ubc-9.

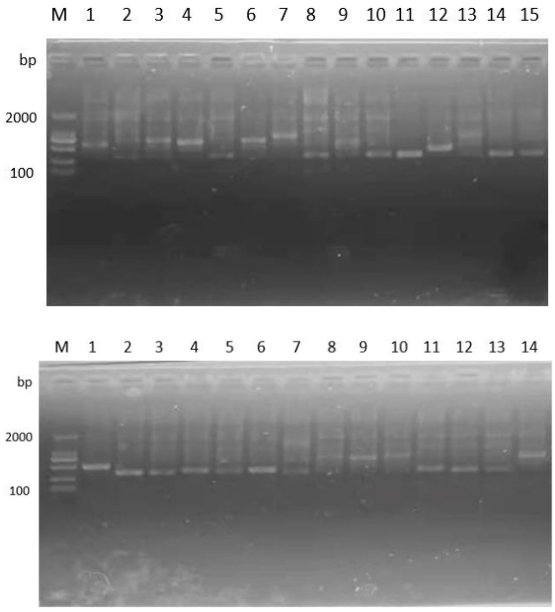

Figure 2. CGSIV DNA amplified by PCR. M:DL2000,1-15:Inserts of CGSIV DNA amplified by PCR

The PCR products of positive colonies in yeast two-hybrid experiment were sequenced by Beijing Genomics institution. Blast the sequence to analyze the result according to NCBI online. Seven genes related to CGSIV were obtained (Table 2). Seven CGSIV protein fragments interacting with Ubc-9 protein in EPC cells were screened by yeast two-hybrid assay, CGSIV 9R, 18L, 30L, 37R, 49R, 50L and 57L.

Table 2. Blast result of Yeast two-hybrid assays

Protein ID	Protein	CGSIV ORF
AHA80853.1	DNA-dependent RNA polymerase largest submit	9R
AHA80862.1	Immediate early protein ICP-46	18L
AHA80893.1	DNA-dependent RNA polymerase b submit	49R
AHA80901.1	Putative SAP domain-containing protein	57L
AHA80881.1	Hypothetical protein	37R
AHA80894.1	DNA polymerase	50L
AHA80874.1	Transcription elongation factor-SII	30L

3.3 Yeast two-hybrid assay

pGADT7-57L and pGBKT7-Ubc-9 were transferred into yeast Y187 and Y2H Gold by PEG3350/LiAC method for yeast two-hybrid experiment. As shown in Figure 3, in the positive control group (pGBKT7-53 + pGADT7-T) and experimental group (pGBKT7-Ubc-9 + pGADT7-57L) were grew positive clones on the plate, while in the negative control group (pGBKT7-Lam and pGADT7-T) did not grow clones. It indicated that Ubc9 of EPC cells could interact with CGSIV 57L protein.

ISSN: 2414-1895 DOI: 10.6919/ICJE.202103_7(3).0037

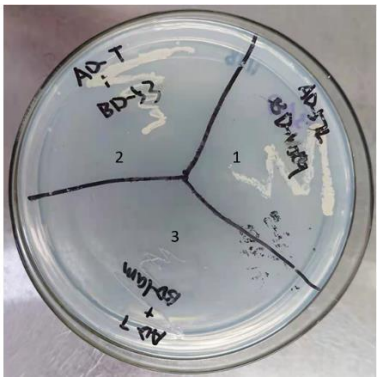

Figure 3. Results of Yeast two-hybrid. 1: pGBKT7-Ubc-9 and pGADT7-57L, 2: pGBKT7-53 and pGADT7-T, 3: pGBKT7-Lam and pGADT7-T

3.4 GST Pull-down assay

The fusion protein of CGSIV 57L-GST with GST tag and Ubc-9 with His tag were expressed by prokaryotic expression system, and the ability of interaction between the two proteins was analyzed by GST pull-down. Western Blot results of GST Pull-down are shown in Figure 4.

Figure 4A is His-tag antibody. Western Blotting results showed that the Ubc-9 protein was present in lane 1, and the size of the Ubc-9-His fusion protein was approximately 21KDa. However, in the control group of lane 2, GST protein did not bind to Ubc-9, so there was no band of Ubc-9-His fusion protein.

Figure 4B is GST-tag antibody. Western Blotting results showed that the CGSIV 57L protein was present in lane 1, and the size of the 57L-GST fusion protein was approximately 79 KDa. indicating that GST-57L fusion protein was successfully hung on the GST microspheres and recognized by GST-tag antibody. There was a band of 6P-1-GST fusion protein at 25 KDa position in lane 2, GST protein used as a control to eliminate non-specific protein binding. The results confirm the interaction between CGSIV 57L and Ubc-9 protein in vitro.

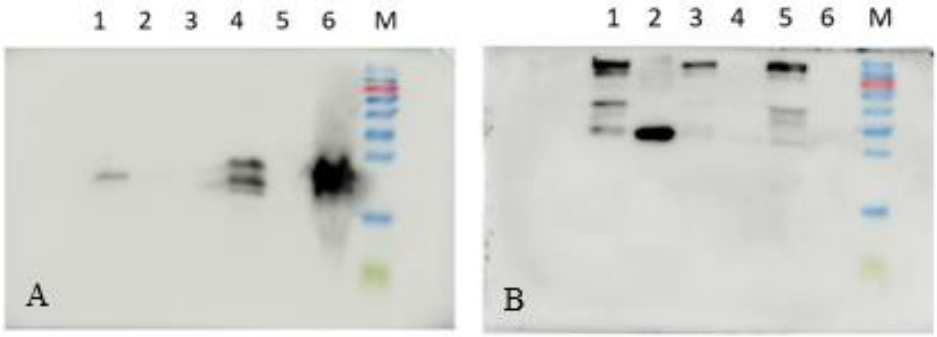

Figure 4. Results of GST Pull-down. 1. GST Resin+supernatant of Ro-pET22b-Ubc-9 and Ro-pGEX6P-1-57L; 2. GST Resin + supernatant of Ro-pET22b-Ubc-9 and pGEX6P-1; 3. supernatant of Ro-pGEX6P-1-57L R; 4. supernatant of Ro-pET22b-Ubc-9; 5. Lysate of Rossetta DE3 containing recombinant p GEX6P-1-57L; 6. Lysate of Rossetta DE3 containing recombinant plasmid pET22b-Ubc-9.

3.5 BiFC identified the interaction between CGSIV 57L and EPC Ubc-9 protein

The plasmids pcDNA3.1-57L-NmCherry and pcDNA3.1-CmCherry-Ubc-9 were transfected into EPC cells. After 48 hours, observe EPC cells under fluorescence microscope. As shown in Figure 5, experimental groups (Figure 5B:57L-NmCherry and CmCherry-Ubc-9) compared with the positive control group (Figure 5A), the red fluorescence is weak, but the negative control group (Figure 5C

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and 5D: 57L-NmCherry + CmCherry and CmCherry-Ubc-9 + NmCherry) did not show red fluorescence. The results showed that EPC Ubc-9 and CGSIV 57L protein could interact in host cells, but the binding ability was weak.

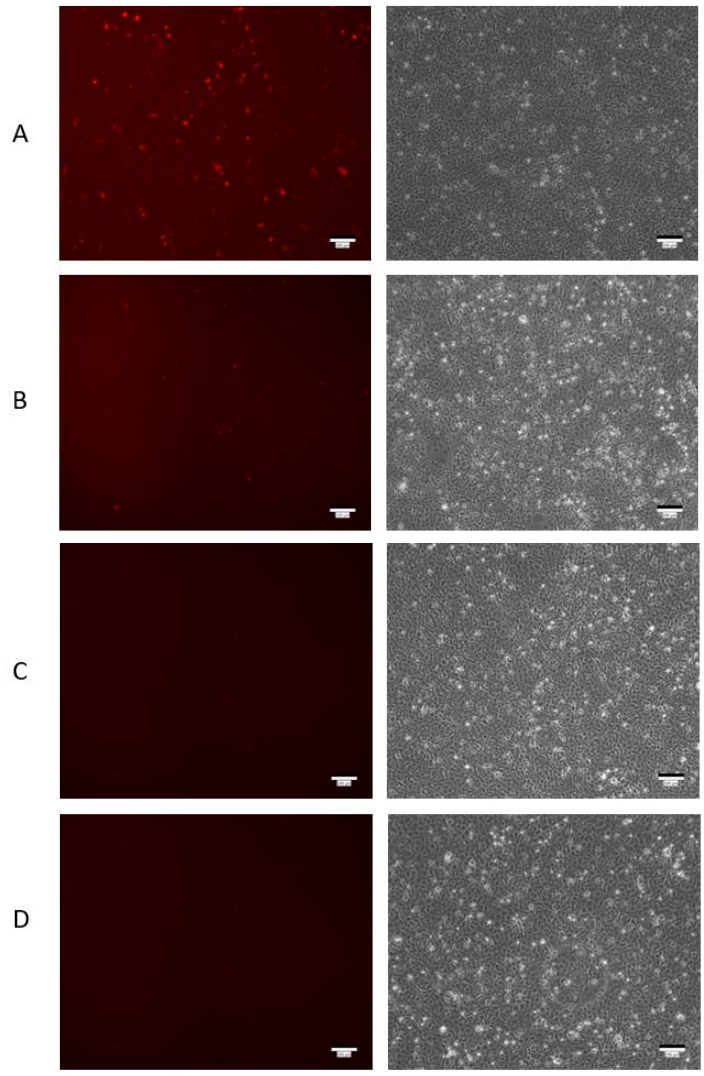

Figure 5. Results of BiFC. A: pcDNA3.1-EGFP-NmCherry+ pcDNA3.1-CmCherry-EGFP. B: pcDNA3.1-57L-NmCherry+ pcDNA3.1-CmCherry-Ubc-9. C: pcDNA3.1-57L-NmCherry+ pcDNA3.1-CmCherry. D: pcDNA3.1-NmCherry+ pcDNA3.1-CmCherry-Ubc-9

4. Conclusion

CGSIV is isolated and identified from diseased Chinese giant salamander (Andrias davidianus), designed as the genus Ranavirus (family Iridoviridae). It have been previously recognized as a major threat to amphibian biodiversity [13]. CGSIV can cause high death rate and result serious great economic losses to breeding industry of Chinese giant salamander [14]. Globally, viral diseases have been acknowledged as a huge threat to the aquaculture industry [15]. Clarifying the mechanism of viral infection and molecular biology is the basis of prevention and treatment of viral diseases. Ranavirus can infect a wide range of aquatic animals, including fish, amphibians and reptiles, which carry a high morbidity and mortality [16]. In order to effectively control the viral diseases of such aquatic animals, a large number of studies have been carried out including viral genome, transcriptome, proteome, viruses and hosts, vaccines and anti-viral drugs. However, there is still a

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lack of in-depth research on important mechanisms, metabolic pathways and genes. In particular, some important mechanisms such as DNA replication, gene transcription, expression regulation and other basic problems have not yet been confirmed and clarified. The research on the interaction and functional mechanism between viral proteins and host proteins lacks the corresponding experimental evidence of biochemistry, molecular biology and virology, which needs more experimental to support. Among them, the relationship between host SUMOylation regulate the activity of viral proteins involve in viral replication is an important to clarify the relationship between virus and host, which is worthy of further study.

In summary, the CGSIV DNA library was constructed in this study, which provided materials for subsequent screening of proteins interacting with viruses. The interaction between CGSIV 57L protein and EPC Ubc-9 was identified, which may provide a clue for exploring the mechanism of virus replication using host SUMO system.

Acknowledgments

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References

[1] Li M, Wang J, Zhang J, et al. First report of two Balantidium species from the Chinese giant salamander, Andrias davidianus: Balantidium sinensis Nie 1935 and Balantidium andianusis n. sp[J]. Parasitology research, 2008, 102(4): 605-611.

[2] Meng Y, Ma J, Jiang N, et al. Pathological and microbiological findings from mortality of the Chinese giant salamander (Andrias davidianus)[J]. Archives of virology, 2014, 159(6): 1403-1412.

[3] King A M, Lefkowitz E, Adams M J, et al. Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses[M]. 9. Elsevier, 2011.

[4] Yan X, Yu Z, Zhang P, et al. The capsid proteins of a large, icosahedral dsDNA virus[J]. Journal of molecular biology, 2009, 385(4): 1287-1299.

[5] Ince I A, Özcan O, Ilter-Akulke A Z, et al. Invertebrate iridoviruses: A glance over the last decade[J]. Viruses, 2018, 10(4): 161.

[6] Chinchar V G, Yu K H, Jancovich J K. The molecular biology of frog virus 3 and other iridoviruses infecting cold-blooded vertebrates[J]. Viruses, 2011, 3(10): 1959-1985.

[7] Jancovich J K, Bremont M, Touchman J W, et al. Evidence for multiple recent host species shifts among the ranaviruses (family Iridoviridae)[J]. Journal of virology, 2010, 84(6): 2636-2647.

[8] Qiu L, Chen X, Zhao R-H, et al. Description of a natural infection with decapod iridescent virus 1 in farmed giant freshwater prawn, Macrobrachium rosenbergii[J]. Viruses, 2019, 11(4): 354.

[9] Müller S, Hoege C, Pyrowolakis G, et al. SUMO, ubiquitin's mysterious cousin[J]. Nature reviews Molecular cell biology, 2001, 2(3): 202-210.

[10] Mattoscio D, Segré C V, Chiocca S. Viral manipulation of cellular protein conjugation pathways: The SUMO lesson[J]. World journal of virology, 2013, 2(2): 79.

[11] Xu M, Wei J, Chen X, et al. Molecular cloning and expression analysis of small ubiquitin-like modifier (SUMO) genes from grouper (Epinephelus coioides)[J]. Fish & shellfish immunology, 2016, 48: 119-127.

[12] Varadaraj A, Mattoscio D, Chiocca S. SUMO Ubc9 enzyme as a viral target[J]. IUBMB life, 2014, 66(1): 27-33.

[13] Duffus A L J, Waltzek T B, Stöhr A C, et al. Distribution and Host Range of Ranaviruses[J], 2015.

[14] Qiya Z, Jian-Fang G. Virus genomes and virus-host interactions in aquaculture animals[J]. Science China, 2015, 58(002): 156-169.

[15] Yu Y, Huang Y, Ni S, et al. Singapore grouper iridovirus (SGIV) TNFR homolog VP51 functions as a virulence factor via modulating host inflammation response[J]. Virology, 2017: S0042682217302027.

[16] Williams T. The Iridoviruses[J]. Advances in Virus Research, 1996, 46: 345-412.