Construction of HSV-1 HF based replication defective vector

Li Xiang, Xinjing Liu, Huitao Liu, Zhiqiang Han, Jiameng Lu and Yuming Xu *

Department of Neurology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan province, 450052, P. R. China. yumingxu@zzu.edu.cn

Abstract: Due to the unique biological features of original virus, HSV-1 derived vectors are rendered a number of advantages, such as broad cell tropism, including infection non-dividing mammalian cells across a broad range of species, and large capacity for foreign gene delivery. To date, two types of recombinant HSV-1 vectors known as replication competent vector and replication defective vector, derived from different HSV-1 strains, such as strain KOS and strain 17 have been developed as gene delivery vehicles and applied in fundamental studies and clinical trials for gene therapy. However, so far, no HSV-1-HF strain derived recombinant vector has been reported yet. Since the features of HSV-1 vectors are largely associated their original virus strains, the HSV-1 vectors from different strains may vary in their infection ability, tropism and cytotoxicity. Here, we described the construction of a HSV-1-HF strain based replication defective vector by a homologous recombination approach in bacteria.
[Li Xiang, Xinjing Liu, Huitao Liu, Zhiqiang Han, Jiameng Lu and Yuming Xu. **Construction of HSV-1 HF based replication defective vector.** Life Science Journal. 2011; 8(3):547-553] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Key words: HSV-1, homologous recombination, replication defective vectors

Introduction

Herpes simplex virus type 1 (HSV-1) is an important human pathogen that causes a variety of diseases from mild skin diseases, such as herpes labialis to life-threatening diseases, such as devastating herpes encephalitis (1-2). Due to its unique features, such as broad cell tropism to infect a wide variety of cell types including the most proliferating and non-dividing mammalian cells across a broad range of species, the large transgene capacity after deleting non-essential genes, and the neurotropic property to infect the nervous system in both anterograde and retrograde directions, HSV-1 derived vectors have been widely used in gene transfer and gene therapy studies and hold promising potential for human disease gene therapy and vaccine application (3). Since it does not integrate into host chromosomes, HSV-1 derived vectors strongly reduce the risk of insertional mutagenesis. To date, two classes of recombinant HSV-1 vectors known as replication competent vector and replication defective vector, derived from different HSV-1 strains, such as strain KOS and strain 17 have been developed as gene delivery vehicles and applied in fundamental studies and clinical trials, including delivery and expression of human genes to cells of the nervous system, selective destruction of cancer cells and prophylaxis against infection with HSV or other infectious diseases (2-3). The replication defective HSV-1-based vectors are herpes viruses in which genes that are essential for viral replication have been either mutated or deleted so as to reduce their cytotoxicity and, together with other deletions involving nonessential genes, have also created space to introduce distinct and independently regulated expression cassettes for different transgenes(3). A replication-defective HSV vector for the treatment of chronic pain, such as inflammatory pain, neuropathic pain and pain caused by cancer in bone, has recently entered in phase 1 clinical trial(3-4) and replication-competent (oncolytic) vectors have reached phase II/III clinical trials. (3). Since the features of HSV-1 vectors are largely associated with the biological character of their original virus strains, the HSV-1 vectors from different strain may vary in their infection ability, tropism and cytotoxicity. Although several replication-defective HSV-1 vectors have been constructed mainly from HSV-1 strain 17+(5), and strain KOS(6-8), however, so far, there is no HSV-1-HF strain derived recombinant vector was reported yet. HSV-1 strain HF is an especially weak pathogenic laboratory strain(9) and has its specific biological characters, such as reduced neurovirulent pathogenicity compared with HSV-1 strains 17 syn+ and KOS and high levels of thymidine kinase activity (10). We previously cloned the whole genome of HSV-1 Strain HF into a bacterial artificial chromosome of F plasmid, named BAC- HSV-1-HF (11), and we here described the construction of a HSV-1-HF based replication defective vector by using of a simple and highly efficient BAC recombineering approach in bacteria (12).

Materials and methods

Cells: African green monkey kidney (Vero) cells, CNE cells (human nasopharyngeal carcinoma epithelioid cell line), HFL-1 cells (human lung fibroblasts strain) and 293A cells (human embryonic kidney cell strain) were purchased from Shanghai

Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. The 2-2 cell line is kindly provided by Dr Jia laboratory in The Prostate Centre at Vancouver General Hospital, Vancouver, British Columbia, Canada. All cells were grown in Dulbecco's Modified Eagle Medium (DMEM), except HFL-1 cells in MEM, supplement with 10% fetal calf serum in humidified 37°C, 5% CO_2 incubator.

Plasmids and bacteria: The plasmid pYD-C255, a GalK-kan (galactokinase-kanamycin) dual-expression cassette and a recombineering E. coli strain SW105 were gifted by Dr Yu, Washington University in St. Louis; The plasmid BAC-HSV-1-HF was constructed by our laboratory as described previously(11), which is able to produce infectious HSV-1 HF virus when transfected it into HSV permissive cells;

Virus: The infectious HSV-1-HF virus were prepared by transfecting vero cells with BAC- HSV-1-HF plasmid DNA

Constructed a replication defective BAC-HSV-1 HF vector:

As shown by a schematic diagram below, we constructed a replication defective BAC-HSV-1 HF vector by using of a simple Red recombination approach(12) in SW105 bacteria in two steps: the first step was to delete the essential IE gene ICP27 by a positive selection to construct a BAC-HSV-1-HF-△ICP27 replication defective vector, which harbored galk-kan selection genes; The next step was to introduce a hTERT-ICP27 cassette into the BAC-HSV-1-HF-△ICP27 replication defective vector by a negative selection and replaced galk-kan sequences simultaneously for engineering a tumor specific replication competent oncolytic virus vector, BAC-HSV-1-HF- hTERT- ICP27.

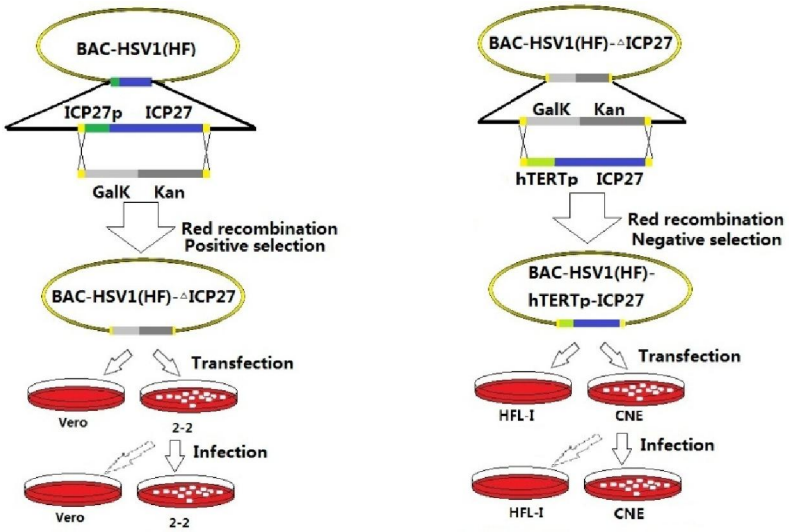

Fig.1. Schematic diagram showing the flowchart of the construction of recombinant BAC-HSV-1-HF-△ICP27 replication defective vector by a positive selection and a tumor specific replication competent oncolytic virus vector, BAC-HSV-1-HF-hTERT-ICP27 by a negative selection.

1. Construction of BAC-HSV-1-HF-△ICP27 replication defective vector by positive selection scheme

To delete the ICP27 gene (DQ889502:103991-106290) from BAC-HSV-1-HF, a Red recombination was applied through galK positive selection scheme in SW105 bacteria strain and the GalK-kan cassette, containing homology arms to the flanking region of UL54 was inserted, by homologous recombination, into the in BAC-HSV-1-HF. The brief procedure is as follows:

1) The GalK-kan primers design and GalK-kan cassette PCR amplification:

GalK-kan primers with 50 bp homology to the area flanking the UL54 site was designed and synthesized at the following sequences: the forward primer 5'-TGGCGCTTCACTACGAGCAGGAGATCCAGAGG CGCCTGTTTGATGTATGACCTGTTGACAATTAA TCATCG-3'and the reverse primer 5'-GTTATGTCCGGGGCCCGTAAGAACAGGTTGG TGAGGGGGGTCGCTGTCATCTCAGCAAAAGTT CGATTTA-3'. PCR amplification 2278 bp fragment of the GalK-kan cassette using the primers designed above, a PrimeSTAR HS DNA Polymerase -mix and 2 ng pYD-C255 plasmid as template at the program of 94°C 15s, then 94°C/8s, 59°C/1m, 68°C 2.5m for 25 cycles and 68°C extension for 3 min. The PCR products were subjected to a digestion by adding 1 ul DpnI into the PCR reaction at 37°C for 3 hours and gel-purified the 2.3 kb PCR product into 50 ul ddH_2O for a transformation.

2) Transduction of the PCR products into BAC-HSV-1-HF recombineering bacteria

To induce a homology recombination, 23 µl of electrocompetent SW105 bacteria harboring BAC-HSV-1-HF was electroporated with 2 ul of PCR product at 1,750V, 6ms. After electroporation of the PCR product, the bacteria were recovered in 1 ml LB for 5 hr at 31°C in 10 ml culture tube in a 31°C shaking water bath and grow on kan+/cm+ plate over night at 31°C in a cabinet-type incubator. After incubation, a few amp^-, kan^+, cm^+ colonies were picked and streaked onto MacConkey + galactose + chloramphenicol indicator plates for another 24 hrs incubation at 31°C and pick a single, bright red (GalK+) colony to inoculate a 5 ml LB +chloramphenicol overnight culture at 31°C.

3) Identification of ICP27 deletion from BAC-HSV1-HF plasmid construct PCR analysis

Three pairs of PCR primers were designed and used to identify the ICP27 deletion from BAC-HSV-HF plasmid construct : UL53-F:ACCTGGTGTTTTGTCTCC and GalK- R TCCTGGGTTTAGTTCCTC, UL53-F and GalK- R were designed to bind to UL53 and GalK sequences respectively and amplify a 329bp fragment .Kan-F GTTGGACGAGTCGGAATC and UL55-R CGCAAAGAAAAAGCAGTG, Kan-F and UL55-R were designed to bind to Kanamycin and UL55 sequences respectively and amplify a 464bp fragment. ICP27 primers: UL54-138-F 5'-GGACGAGGACATGGAAGA-3 and UL54-500-R 5'-GGTTGCGATTGGTTCTGG-3', UL54-138-F and UL54-500-R were designed to bind to Kanamycin and UL55 sequences respectively and amplify a 380bp fragment.PCR was performed by using the Plasmid DNA extracted from positive recombinant clones as templates and the primers above at the program of 94°C for 5min for initial denaturation, 94°C for 30sec, 55°C for 30sec, and 72°C for 30sec in 30 cycles, and a final extension step at 72°C for 7min.PCR products were subjected to a 1% agarose gel separation.

4) Functional analysis to test infective BAC-HSV1-HF-△ICP27 vector virus generation in Vero cells and 2-2 cells

The BAC-HSV-HF-△ICP27 plasmid was extracted from PCR identified positive clone with the Qiagen Plasmid Mini Kit (25) (Cat.No.12123) followed the manufacturer's instruction. Vero cells and 2-2 cells (1.2×10^5 cells/well) grown on the 6-well plate were transfected with 2 µg of BAC-HSV-HF-△ICP27 plasmid DNA using lipofectamine 2000 following the manufacturer's protocol. The virus was harvested 5 to 7 days after transfection, when CPE was observed, by three freeze/thaw cycles and sonication of collected cells. Recombinant viruses were used to infect the Vero cells again to test the virus production by observation GFP fluorescence.

2. Engineering the foreign gene(s) or cassette(s) of interest into the recombinant BAC-HSV-1-HF-△ICP27replication defective vector

To engineer the foreign gene(s) or cassette(s) of interest into the recombinant BAC-HSV-1-HF-△ICP27replication defective vector and test the function of the vector, we introduced a PCR product containing a hTERT promoter driven ICP27 cassette with the same flanking homology sequences to replace the GalK-kan cassette into the vector by a negative selection scheme as the following procedure.

1) hTERT promoter cloning: Clone of hTERT promoter

PCR to amplify hTERT promoter of genome was performed with the primers of forward: 5'-TTTGGATCCCGATTCGACCTCTCTCCGCTGGGGC-3'and reverse: 5'-TTTCTCGAGCAGGGCTTCCCACGTGCGCAGCAG -3' for a 410bp band by using of the 293A cell genomic DNA as a template with the condition of : 94°C for 5min for initial denaturation, 94°C for 30sec, 59°C for 30sec, and 72°C for 30sec in 30 cycles, and a final extension step at 72°C for 7min. The PCR products were cloned to pMD18-T vector via TA cloning method. Plasmid pMD-hTERTp extracted from positive colonies was sequence confirmed.

2) ICP27 gene cloning: Clone of ICP27 gene

PCR amplification of ICP27 gene from HSV-1 genome was performed with the primers: forward, 5'-CGACAGCTCTGAAATGGCGACTGACATTGATATGC-3'and reverse: 5'-GTTTTGCGGCCGCTAAATCCGTCCCCGTTCC-3' for a 1.7K band by using of the HSV-1(HF) virus stock as a template with the condition of : 94°C for 5min for initial denaturation, 94°C for 30sec, 63.2°C for 30sec, and 72°C for 2min in 30 cycles, and a final elongation step at 72°C for 7min. PCR production was cloned to pGM-T vector via TA cloning method. The pGM-ICP27 DNA extracted from the correct clone was sequenced.

3) Construction of The plasmid pMD-hTERTp-ICP27

The plasmid pMD-hTERTp-ICP27 was subject to serially digestion and gel purification in the following order: pMD-hTERTp, digested by XhoI to get linear DNA, blunt by Klenow fragment and dephosphorylated by FastAP, then was ligated with the 1.7 kb ICP27 fragment acquired from pGM-ICP27, digested by EcoRI and blunted by Klenow fragment. The plasmid pMD18-hTERTp-ICP27 was restriction analyzed to identify the correct clone.

4) PCR amplification of hTERT promoter driven

ICP27 expression cassette
PCR primers were designed with 5'ends same as the homology arms used in the ICP27 knock out step and 3'ends binding to the upstream sequence of hTERT promoter and downstream sequence of ICP27 expression cassette respectively: 5'-TGGCGCTTCACTACGAGCAGGAGATCCAGAG GCGCCTGTTTGATGTATGA CGATTCGACCTCTCTCCGCTGGGGC -3' and the reverse primer: 5'-GTTATGTCCGGGGCCCGTAAGAACAGGTTGG TGAGGGGGGTCGCTGTCATTAAATCCGTCCCCG TTCC-3'. PCR amplification of 2.2 KB fragment was performed at the program of 94°C 15S, then 94°C/8s, 59°C/1m, 68°C 2.5m for 25 cycles and 68°C extension for 3 min by using pMD18-hTERTp-ICP27 plasmid as the template. The procedure of PCR products Dpn1 digestion, gel purification, electroporation in the bacteria of BAC-HSV-1HF-△ICP27-galk was same as described above.

5) Recombinant colonies selection
the recovered bacteria from electroporation were washed twice with 1ml 1× M9 salts and the pellet at 13,200 rpm/15s, was re-suspend, Plated on cm^+ galk negative selection plates and Incubated at 31°C for 2 days until small colonies grow out. The colonies grown on Kan plate (negative) & cm^+ plate were picked and the BAC DNA was prepared for restriction digestion, PCR and functional analysis.

6) PCR analysis
PCR was carried out to identify the recombinant BAC-HSV1-HF-hTERT-ICP27 clones by the same primers and conditions as used in positive selection step except templates by using the plasmid DNA from selected colonies from the negative selection.

7) Functional analysis by testing infective virus generation in HFL-I and CNE cells
The BAC-HSV1-HF-hTERT-ICP27 plasmid was extracted from selected PCR identified correct clone with the Qiagen Plasmid Mini Kit (25)(Cat.No.12123) followed the manufacturer's instruction. HFL-I and CNE (1.2×10^5 cells/well) plated in 6-well plate were transfected with 2 μg of BAC-HSV1-HF-hTERT-ICP27 plasmid DNA using lipofectamine 2000 respectively following the manufacturer's protocol. The virus were harvested 5 to 7 days after transfection, when CPE was observed, by three freeze/thaw cycles and sonication of collected cells. Recombinant viruses were used to infect the HFL-I and CNE again to determine the virus generation by observation GFP fluorescence.

Results:

1. Construction of BAC-HSV-1-HF-ΔICP27

replication defective vector by positive selection

Through galK positive selection of a Red recombination scheme in SW105 bacteria strain, we obtained recombinant bacteria colonies, which were grown on MacConkey + galactose + chloramphenicol indicator plates. In Figure 2, recombinant bacteria colonies were shown in bright red color.

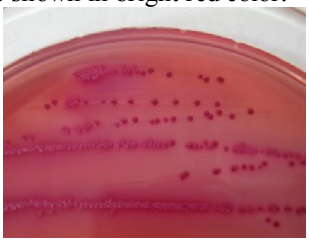

Fig. 2. Recombinant bacteria colonies showing in bright red color on MacConkey + galactose + chloramphenicol indicator plates

PCR analysis of galK-kan gene and ICP27 form the selected recombinant clones showed the ICP27 gene was deleted from BAC-HSV-1-HF genome. In Figure 3, agarose gel electrophoresis showed the correct PCR products of galK-kan gene with the primers of UL53F/GalR in A, KanF/UL55R in B and ICP27 F/R in C.

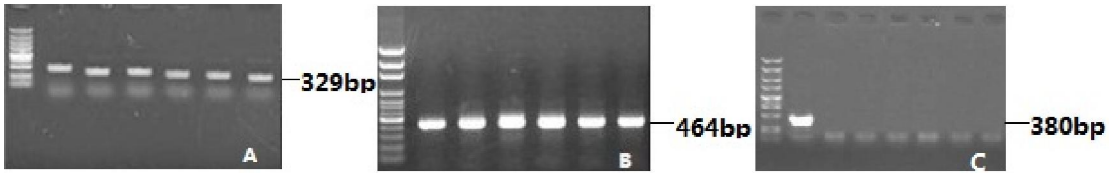

Fig. 3. Agarose gel electrophoresis showing a 329bp band of UL53F/GalR PCR product of galK gene in A, a 464bp band of KanF/UL55R PCR product of Kanamycin gene in B and 380bp of ICP27 F/R PCR product of ICP27 in C.

Identification of ICP27 deletion from BAC-HSV-HF plasmid constructs:Functional analysis to test infective BAC-HSV-HF-△ICP27 vector virus generation in Vero cells and 2-2 cells. After transfection of the Vero cells and 2-2 cells with BAC-HSV1-HF-△ICP27 plasmid DNA from PCR

identified recombinant clone, CPE was observed in 2-2 cells, but not in Vero cells. As shown in Figure 4, When infected the Vero cells and 2-2 cells with the harvested supernatants from the transfected Vero cells and 2-2 cells, the CPE and GFP fluorescent was only observed in the cells infected by the harvested supernatants from 2-2 cells. Wheras a parallel positive control, transfection with BAC-HSV1-HF plasmid DNA was observed CPE and GFP fluorescent only in the transfection step and neither the CPE and GFP fluorescent in the infection step always. These results indicated that ICP27 gene was successfully deleted.

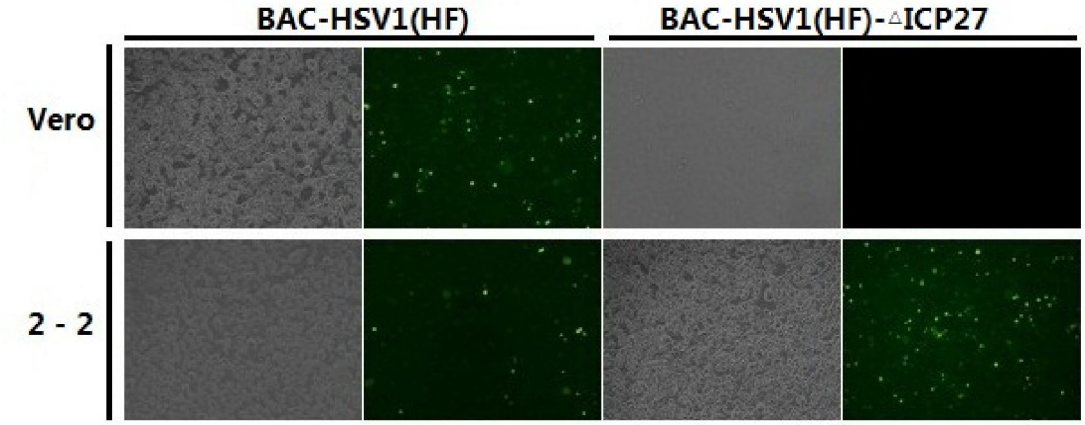

Fig.4. CPE and GFP fluorescence observation in Vero cells and 2-2 cells infected with BAC-HSV1-HF plasmid in the left panel and BAC-HSV1-HF-△ICP27 plasmid in the right panel.

The CPE in the transfection step with BAC-HSV1-HF-△ICP27 plasmid and GFP fluorescence in the infection step with harvested supernatant from the transfection step were only observed in 2-2 cells, but not in Vero cells, while in a positive control with BAC-HSV-HF plasmid, CPE and GFP fluorescence were observed in both Vero cells and 2-2 cells.

Based on the results obtained above, we concluded that a replication defective vector BAC-HSV-HF-△ICP27 was constructed.

2. Engineering the a hTERT promoter driven ICP27 cassette into the recombinant BAC-HSV-1-HF-△ICP27 replication defective vector

To test the function of recombinant BAC-HSV-1-HF-△ICP27 replication defective vector, we introduced a hTERT promoter driven ICP27 cassette into the vector by a negative selection to engineer a BAC-HSV-1-HF-hTERT-ICP27.

By PCR amplifications, we successfully obtained a 410 bp hTERT promoter element and a 1.7Kb ICP27 element and cloned them into a pMD-18T vector to result a hTERT promoter driven ICP27 cassette. The agarose gel electrophoresis showed the correct size of the PCR products sequence confirmed of hTERT promoter and ICP27 element gene structure(Data not show). The hTERT promoter driven ICP27 cassette with the same homology arm of ICP27 deletion, was amplificated by PCR and electro transformated into the BAC-HSV1-HF-△ICP27 competent bacterial.

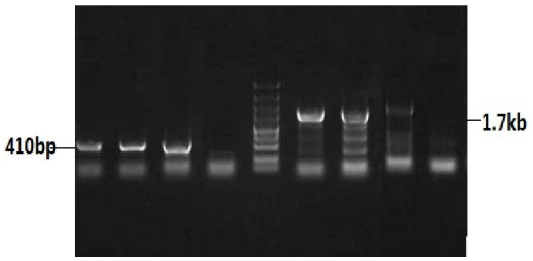

Fig. 5. Agarose gel electrophoresis analysis BAC-HSV1-HF-hTERT-ICP27, showing a 410bp band of hTERT promoter PCR fragment and a 1.7K band of ICP27 gene PCR fragment in respectively.

PCR analysis showed recombinant colonies carrying hTERT promoter and ICP27 sequences, indicating hTERT-ICP27 cassette was successfully engineered into BAC-HSV-HF-△ICP27 vector, , As shown in fig.5. Through a negative selection, the recombinant colonies grown on Kan plate (negative) & cm (positive) plate were obtained. This Identificated the absent of the galK-Kan elements. The recombinant bacteria have lost the galK-kan cassette by a deletion, and the rest will be truly recombinant clones.

After transfection of HFL-I and CNE cells with the plasmid DNA BAC-HSV1-HF-hTERT-ICP27 extracted from recombinant clone, the CPEs and GFP fluorescence were observed only in CNE cells, but not in HFL-I cells, indicting the infective virus were restricted in the tumor cells. To determine if the infective virus were selectively generated in tumor cell specific manner, we thus collected the supernatant from

the transfected cells and infected the HFL-I and CNE cells respectively again and took the CPE and GFP fluorescence observation. After infection, as shown in Figure 6, the CPE and GFP fluorescence were restrictively observed CNE cells, but still not in HFL-I cells indicating the infective virus were specifically generated in tumor cells. As a parallel negative control, BAC-HSV1-HF-△ICP27 failed to generate any CPE after transfection and nor infective GFP fluorescence after infection with harvested supernatant. These results suggested that recombinant BAC-HSV1-HF-hTERT-ICP27 is tumor selective replication competent oncolytic virus.

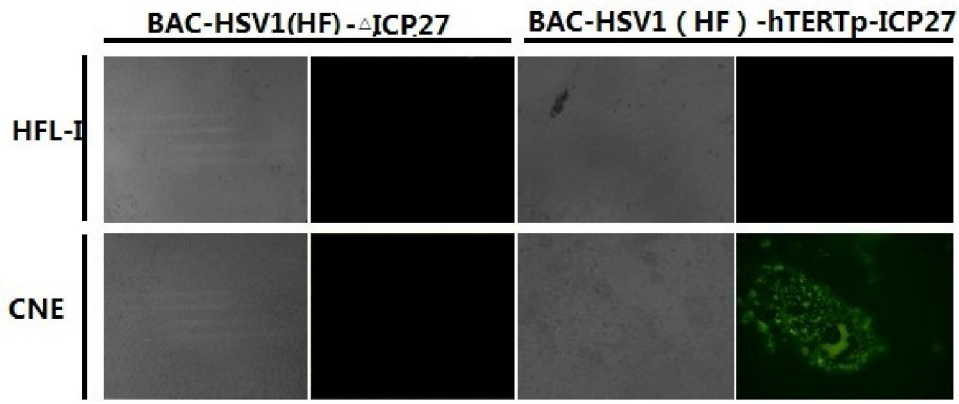

Fig.6. CPE and GFP fluorescence observation in HFL-1 and CNE cells, which were treated with the supernatants from corresponding cells transfected with BAC-HSV1-HF-△ICP27 and BAC-HSV1-HF-hTERT-ICP27 vector.

Discussions

Utilizing a red homologous recombination technique in bacteria, we successfully constructed a BAC-HSV-1HF based recombinant replication defective vector by deleting ICP27 and leaving a GalK-Kan cassette in UL54 locus for foreign gene clone selection, which enabled us to develop a tumor-specific replication competent oncolytic virus for cancer therapy studies. Red homologous recombination technique is rapid and high efficient novel recombination technique, which makes it simple and easy to knock out, mutant or engineer almost any foreign gene(s) or cassette(s) into the BAC plasmid carried large DNA molecule, including the whole genome of HSV-1 virus compared with traditional homologous recombination techniques in mammalian cells. We used a two step scheme to delete the essential IE gene UL54, encoding ICP27 protein, by a galk-Kan cassette instead and to construct a replication defection vector, which allowed foreign gene cassette(s) to be engineered into the vector by a simply negative selection. Our recombinant HSV replication defective vector has potential for development of both tumor-specific replication competent Oncolytic HSV virus and gene delivery vectors for gene therapy of different diseases. Although different oncolytic virus have been developed and applied to preclinical and clinical studies, for example, the first-Generation HSV Vectors were designed to mutant a single gene so as to restrict their replication in dividing cells, the second Generation HSV Vectors was resulted in multigenic mutations, and the third-Generation Vectors was designed to delete some essential genes plus US12 gene, encoding ICP47 protein to block MHC class I-mediated antigen presentation, for immune-modulation, however, the last Generation Vectors would be designed to develop the transgene-expressing vectors so as to further augment their antitumor efficacy by incorporation of expression cassettes for the delivery of various transgenes (3). By using this strategy, Dr. Jia et al 2010 developed a novel targeting replication HSV-1 viruses by dual-regulating viral essential gene expression of ICP27 in both transcriptional and translational levels to increase viral lytic activity and tumor specificity by systemic treatment advanced and metastatic prostate cancers(13). Therefore, the development of conditional replication competent HSV-1 viruses armed with multiple transgenes might present the future direction in oncolytic virotherapy. HSV-1 strain HF is a laboratory strain and has been demonstrated to have especially weak pathogenic via a intravaginal inoculation (9). Compared with HSV-1 strains 17 syn+ and KOS it exhibited reduced neurovirulent pathogenicity in mouse brains, although could also produce a lethal infection, but was completely avirulent after footpad inoculation (10). In addition, HSV-1 strain HF was also demonstrated to have high levels of thymidine kinase activity. As HSV-1 HF derivative clone 10 (HF10), a spontaneously occurring, highly attenuated virus of HSV-1-HF, was demonstrated to have strong anti-tumor activity both in animal model

studies and clinical trial (14-15), therefore, our BAC-HSV-1-HF-ΔICP27 replication defective vector would have a promising potential to be modified by engineering method to acquire less neurovirulent and more efficient oncolytic effects than natural mutant HF10 to be used for cancer therapy. Furthermore, RNA interference is a recently developed novel gene silencing technique and has been demonstrated to be able to efficiently knock down targeting gene by both synthetic small interfering RNAs (siRNAs) and expressed small hairpin RNA (shRNA) in many cell types, including in neurons, however, in vivo delivery of RNAi remains a major challenge, thus limiting its applications. Recently, replication-defective herpes simplex viral (HSV-1) vectors have been demonstrated to be a highly efficient method for in vivo gene silencing in dorsal root ganglia (DRG) using HSV-mediated delivery of short-hairpin RNA (shRNA) targeting reporter genes (16). Therefore, our BAC-HSV-1HF based recombinant replication defective vector might be an ideal shRNA delivery vehicle for gene silencing in nerve system.

Conclusion

We have for the first time constructed a herpes simplex virus type 1 strain HF based recombinant replication-deficient vector, in which the UL54 gene locus was replaced by galk-Kan cassette, thus making it easy to introduce any gene(s) of interest into the vector by a simple negative selection. Our HSV-1 HF based replication defective vector might be ideal platform for gene delivery, oncolytic virotherapy and vaccine development as well.

Corresponding author: Dr. Yuming Xu, yumingxu@zzu.edu.cn. Mailing address:1 East Jianshe Road，Zhengzhou, Henan province, 450052, P. R. China

References

1. Watanabe D. Medical application of herpes simplex virus. J Dermatol Sci. 2010 Feb;57(2):75-82.
2. Fraefel C, Marconi P, Epstein AL. Herpes simplex virus type 1-derived recombinant and amplicon vectors. Methods Mol Biol. 2011;737:303-43.
3. Manservigi R, Argnani R, Marconi P. HSV Recombinant Vectors for Gene Therapy. Open Virol J. 2010 4:123-56.
4. Wolfe D, Mata M, Fink DJ A human trial of HSV-mediated gene transfer for the treatment of chronic pain. Gene Ther. 2009 Apr;16(4):455-60.
5. Krisky DM, Marconi PC, Oligino TJ, Rouse RJ, Fink DJ, Cohen JB, Watkins SC, Glorioso JC. Development of herpes simplex virus replication-defective multigene vectors for combination gene therapy applications. Gene Ther. 1998 Nov;5(11):1517-30.
6. Wu N, Watkins SC, Schaffer PA, DeLuca NA.Prolonged gene expression and cell survival after infection by a herpes simplex virus mutant defective in the immediate-early genes encoding ICP4, ICP27, and ICP22. J Virol. 1996 Sep;70(9):6358-69.
7. DeLuca NA, McCarthy AM, Schaffer PA. Isolation and characterization of deletion mutants of herpes simplex virus type 1 in the gene encoding immediate-early regulatory protein ICP4. J Virol. 1985 Nov;56(2):558-70.
8. McCarthy AM, McMahan L, Schaffer PA. Herpes simplex virus type 1 ICP27 deletion mutants exhibit altered patterns of transcription and are DNA deficient. J Virol. 1989 Jan;63(1):18-27.
9. Nishiura H, Hayakawa K, Matsui Y, Sawada M, Nii S. Experimental studies on genital herpetic infection in mice. Biken J. 1980 Dec;23(4):169-77.
10. Sedarati F, Stevens JG. Biological basis for virulence of three strains of herpes simplex virus type 1. J Gen Virol. 1987 Sep;68 (Pt 9):2389-95.
11. Liu X, Song B, lu J, Wang Q, Han Z, Xu Y. The Construction of BAC-HSV-1 Strain HF with GFP Reporter Gene and the research of Its Infectious Progeny Virus. Chinese journal of virology, 2011; 27（3）238-243.
12. Warming S, Costantino N, Court DL, Jenkins NA, Copeland NG. Simple and highly efficient BAC recombineering using galK selection. Nucleic Acids Res. 2005 Feb 24;33(4)
13. Lee CY, Bu LX, DeBenedetti A, Williams BJ, Rennie PS, Jia WW. Transcriptional and translational dual-regulated oncolytic herpes simplex virus type 1 for targeting prostate tumors. Mol Ther. 2010 May;18(5):929-35.
14. Takakuwa H, Goshima F, Nozawa N, Yoshikawa T, Kimata H, Nakao A, Nawa A, Kurata T, Sata T, Nishiyama Y. Oncolytic viral therapy using a spontaneously generated herpes simplex virus type 1 variant for disseminated peritoneal tumor in immunocompetent mice. Arch Virol. 2003 Apr;148(4):813-25.
15. Mori I, Liu B, Goshima F, Ito H, Koide N, Yoshida T, Yokochi T, Kimura Y, Nishiyama Y. HF10, an attenuated herpes simplex virus (HSV) type 1 clone, lacks neuroinvasiveness and protects mice against lethal challenge with HSV types 1 and 2. Microbes Infect. 2005 Dec;7(15):1492-500.
16. Anesti AM, Peeters PJ, Royaux I, Coffin RS. Efficient delivery of RNA Interference to peripheral neurons in vivo using herpes simplex virus. Nucleic Acids Res. 2008 36(14)

9/20/2011