Construction and identification of a recombinant adenovirus vector expressing His-tag-ICP47 fusion gene

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Abstract: Background: The response of host immune systems against gene products expressed by genetically modified cells is a major obstacle to successful gene therapy. Major histocompatibility complex (MHC) class Ⅰ antigen presenting pathway is very important in acute allograft rejection and blocking MHC Ⅰ antigen expression is becoming a research hotspot of inducting immune tolerance. Infected cell protein 47 (ICP47) expressesd by herpes simplex virus type 1 (HSV-1), inhibits MHC Ⅰ antigen presentation pathway by binding to host transporter associated with antigen presentation (TAP), and thereby reduces the rate of cytolysis of infected cells by sensitized CTL and evades the host immune clearance. The aim of this study is to construct and identify a recombinant adenovirus expressing His-tag-ICP47 fusion gene for the following studies of its immunological activities. **Methods:** The adenoviral vector system of AdEasy-1was used to prepare the recombinant adenovirus expressing His-tag-ICP47 fusion gene or the control empty recombinant adenovirus r-Track. His-tag-ICP47 fusion gene was firstly cloned into the pAdTrack-CMV vector. The gene fragments digested by Pme Ⅰ were co-transformed with adenoviral backbone vector pAdEasy-1 in E.coli BJ5183 cells to produce recombinant adenoviral vector pAdEasy-H-ICP47. Linearized with *Pac Ⅰ*, recombinant adenoviral vector was subsequently transfected into 293 cells to product r-H-ICP47. Meanwhile, the control empty recombinant adenovirus r-Track was built in the same way. Finally, The viruses were amplified and virus particle titres were determined. Purified virus was analyzed for the presence and expression of His-tag-ICP47 fusion gene by PCR and Western-blot analysis. **Results:** The recombinant adenoviruses of r-H-ICP47 and r-Track were successfully constructed and successfully transfected into 293 cells and virus granules appeared. The virus particle titres of r-H-ICP47 and r-Track were determined with the resulting of 3.7×10^{10}efu/mL and 4.4×10^{10}efu/mL The proteins producted by recombinant adenovirus-infected cells were confirmed by PCR and Western-blot analysis. **Conclusion:** The replication-defective recombinant adenoviruses of r-H-ICP47 and r-Track are successfully constructed, and the virus particle titres are highly enough to infect and express relevant genes in cells at a desired level, respectively.
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Key Words: recombinant adenovirus; infected cell protein 47; fusion gene

Introduction

Adenoviral vector is considered a safe and efficient way to introduce foreign genes into several kinds of cells and is widely used in the various fields of gene therapy [1]. But the response of host immune systems against gene products expressed by genetically modified cells is a major obstacle to successful gene therapy[2]. Immuno-suppressants are not always effective and associated with well-known toxic effects [3-4]. Therefore, prevention of host immune response against transplanted cells and products of introduced genes could be a critical subject for the long-term success of gene therapy[5].

In particular, major histocompatibility complex (MHC) class Ⅰ antigen presenting pathway is very important in acute allograft rejection and has been an attractive target for immune rejection, and blocking MHC Ⅰ antigen expression is becoming a research hotspot of inducting immune tolerance[6]. Infected cell protein 47 (ICP47), an immediate-early protein expressed by herpes simplex virus type 1 (HSV-1), inhibits MHC Ⅰ antigen presentation pathway by binding to host transporter associated with antigen presentation (TAP), and thereby reduces the rate of cytolysis of infected cells by sensitized CTL and evades the host immune clearance[7-8].

Based on these studies，this subject was designed to construct a recombinant adenovirus vector expressing His-tag-ICP47 fusion gene and identify its immunological activity. We expect this finding should have important implications for analyzing the mechanisms of immune tolerance as well as human gene therapy.

METHODS

DNA manipulation and PCR amplification

The template DNA of ICP47 was extracted from HSV-1-positive CSF specimens (Axygen Biosciences CO., Ltd), ICP47 primers were: sense 5′-GGCAAGCTTATGTCGTGGGCCCTGGA-AATG-3′, antisense 5′-GGCCGATATCTCAACGGGTTACCGGATTACG ‐ 3′, yielding a 286bp. The restriction sites of *Hind Ⅲ* and *EcoR V* were added at the 5' termini of forward and reverse primers, respectively.

PCR amplification was subsequently carried out for the target sequences by using a S1000 PCR cycler (Bio-Rad) in 200μl PCR tubes containing the following reaction mixture: 5 μl template DNA, 5 μl Pfu DNA Polymerase 10× Buffer, 0.5μl Pfu DNA Polymerase (Promega Corporation), 4 μl dNTPs (2.5 mM), and 20 pmol each primer in 50 μl final volume. The amplification cycling profile was as follows: initial denaturation 95°C for 2 minutes; cycling: 95°C for 30 seconds, 60°C for 30 seconds and 72°C for 2 minute (35 cycles); final extension at 72°C for 5 minutes. After reaction completion, 5 μl of PCR products was run on 1.5% agarose gel electrophoresis stained with ethidium bromide, and then, images were scanned by a Transilluminator JY04S Gel analysis system under UV light (Beijing Junyi-Dongfang electrophoresis Equipment CO., Ltd). Finally, PCR products were recovered and purified.

Construction of Adenovirus Vector pAd-H-ICP47

Purified ICP47 DNA segments and pAdTrack-CMV vector were digested with restriction endonuclease *Hind Ⅲ* and EcoR V (New England Biolabs), respectively. Recovered products was connected with *T4* DNA ligase (New England Biolabs), and then the ligation mixture was transformed into DH5α chemically competent cells. Kana-resistant transformants were selected by plating the transformation mixture on LB agar plates supplemented with 50μg/mL Kana. After incubation overnight at 37°C, the recombinant plasmid DNA was isolated and further confirmed via *Hind Ⅲ* and *EcoR V* double digestion, Finally, the confirmed recombinant adenoviral vector was named pAdTrack-ICP47.

A segment containing a 6×His tag was synthesized and directionally inserted pAdTrack-ICP47 at the *Kpn I* and *Hind*Ⅲ sites, resulting in pAdTrack-H-ICP47. After identified with enzyme digestion assay, the His-tag-ICP47 fusion gene nucleotide sequence of final constructs was determined to confirm that no mutation was introduced by the amplification step.

Homologous recombination of pAdEasy-H-ICP47

pAdTrack-H-ICP47 was linearized by restriction endonuclease *Pme I* (New England Biolabs), and subsequently was co-transformed with adenoviral backbone vector pAdEasy-1 in E.coli BJ5183 cells. Kana-resistant transformants were selected by plating the transformation mixture on LB agar plates supplemented with 50μg/mL Kana. Positive clones were selected and enzyme identified, and the recombinant adenoviral vector was obtained and named pAdEasy-H-ICP47. Meanwhile, the control empty recombinant adenoviral vector pAdEasy-Track was constructed in the same way.

Package of recombinant adenovirus

Human embryonic kidney 293 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100μg of penicillin/ml, and 100 U of streptomycin/ml. 293 cells were cultured and plated at a density of 1×10^6 cells/ml for 24 h before transfection, and cells were transfected at about 70% confluence on the next day. pAdEasy-H-ICP47 was digested by *Pac I* (New England Biolabs, Inc) and transfected into 293 cells with Lipofectamine 2000 (Invitrogen) according to the transfection agent instruction to generate adenovirus r-H-ICP47. Seven-ten days later, green fluorescence could be seen under a fluorescence microscope. The supernatant and cells were collected and repeatedly freezed-thawed three times at 37°C/-80°C. The supernatant containing virus was taken in centrifugation and stored at -80°C. Similarly, the control empty recombinant adenovirus r-Track was generated.

Amplification, concentration and purification of recombinant adenovirus

Amplified, concentration and purification of the recombinant adenovirus were carried out as previously described [9], Briefly, the recombinant adenoviruses -infected cells were harvested and washed three times with PBS, and the virus particles were released from the 293 cells by three freeze-thaw cycles and purified by ultracentrifugation on a cesium chloride (CsCl) step gradient and dialysis in the buffer containing 5% sucrose, 10 mM Tris pH 8.0 and 2 mM $MgCl_2$.

Determination of virus particle titres

Purified virus particle titres were determined by counting the number of green fluorescence protein (GFP)-positive cells as previously described[9]. Briefly, 293 cells of a final density of 1×10^5 cells/mL were infected by recombinant adenovirus in a 12-well dish with 10μL aliquots of 1:10 geometric dilution. After 24 h of incubation in 5% CO_2 at 37°C, the GFP-positive cells were counted with a fluorescent light microscope, and the titer was defined in expression- forming units per milliliter (efu/mL) as follows: (efu/mL =number of GFP-positive cells$\times10^{n+1}$/mL).

PCR Analysis

To test whether the His-tag-ICP47 fusion gene were preserved in transduced target cells, total DNA was prepared by lysing transfected 293 cells with 400 mg of proteinase K per milliliter at 55°C for 1 h , boiling for 5 min and then centrifuging to pellet cell debris. PCR amplification was subsequently carried out as described previously. The oligonucleotide primers specific to His-tag-ICP47 fusion gene sequence were as follows:

Sense 5'-ATGCATCATCATCATCATCATA-3' , antisense 5′-GGCCGATATCTCAACGGG-TTACCGGATTACG‐3′, yielding a 304bp. After reaction was completed, 5 μL of the amplified DNA fragments was analyzed by agarose gel electrophoresis.

Western blot analysis

Expression of the proteins produced by recombinant adenovirus was analyzed by Western blot analysis. In brief, the total proteins were extracted from 293 cells infected by r-H-ICP47 or r-Track, and separated by SDS-PAGE on 0.1% SDS in 10% polyacrylamide gels. At the end of the run, polypeptide bands in the gel were electrophoretically transferred to a PVDF membrane (Bio-Rad). The membrane was incubated at room temperature for 1 h with rabbit anti-β-tubulin antibody, rabbit anti-GFP antibody, or rabbit anti-6×His antibody (Bioss Inc.), respectively. The binding of antibody to the specific protein band on the membrane was detected with horseradish peroxidase-conjugated goat anti-rabbit IgG (Bioss Inc.) and was analyzed with an ECL Western blotting detection system (Beyotime Institute of Biotechnology).

RESULTS

Identificantion of pAdTrack-ICP47 with endonuclease digestion assay

The results of electrophoresis showed that there were two bands at approximately 267bp and 9200bp after pAdTrack-ICP47 was digested with *Hind Ⅲ* and *EcoR V*. The both bands were in the right range consistent with the expected length. On the contrary, there was only one band at approximately 9200bp after the control empty adenoviral vector pAdTrack-CMV was digested with *Hind Ⅲ* and *EcoR V*. Similarly, one band was detected at approximately 9200bp after pAdTrack-ICP47 was digested with *Hind Ⅲ* or *EcoR V*, respectively. The result of the recombinant shuttle plasmid pAdTrack-ICP47 was identified by endonuclease digestion of *Hind Ⅲ* and *EcoR V* was shown in Fig. 1.

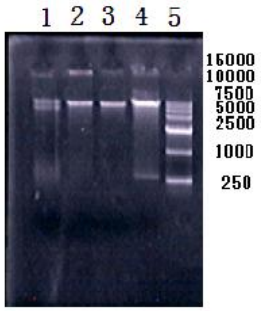

Fig. 1. Identification of pTrack-ICP47 by endonuclease digestion with *Hind Ⅲ* and *EcoR V*

Lane l：pTrack-ICP47；Lane 2：pTrack-CMV/ *Hind Ⅲ* and *EcoR V*；Lane 3：pTrack-ICP47/ *Hind Ⅲ* or *EcoR V*；Lane 4：pTrack-ICP47/ *Hind Ⅲ* and *EcoR V*；Lane 5：DNA Marker DL15000.

Identificantion of pAdTrack-H-ICP47 with endonuclease digestion assay

There were two bands at approximately 294bp and 9200bp after pAdTrack-H-ICP47 was digested with *Kpn I* and *EcoR V*. The both bands were in the right range consistent with the expected length. On the contrary, there was only one band at approximately 9200bp after the control empty adenoviral vector pAdTrack-CMV was digested with *Kpn I* and *EcoR V*. Similarly, one band was detected at approximately 9200bp after pAdTrack-H-ICP47 was digested with *Kpn I* or *EcoR V*, respectively. The result of the recombinant shuttle plasmid pAdTrack-H-ICP47 was identified by endonuclease digestion of *Kpn I* and *EcoR V* was shown in Fig. 2.

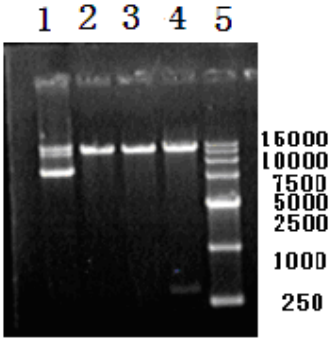

Fig. 2 Identification of pTrack-H-ICP47 by endonuclease digestion with *Kpn I* and *EcoR V*

Lane l：pAdTrack-H-ICP47；Lane 2：pAdTrack-CMV/ *Kpn I* and *EcoR V*；Lane 3：pAdTrack-H-ICP47 / *Kpn I* or *EcoR V*；Lane 4：pAdTrack-H-ICP47 / *Kpn I* and *EcoR V*；Lane 5：DNA Marker DL15000.

Antisense-sequencing identification of pAdTrack-H-ICP47

The result of antisense-sequencing identification of pAdTrack-H-ICP47 was shown in Fig. 3（from site 57 to site 350, 294bp）. Sequencing results verified that His-tag-ICP47 fusion gene fragment had been correctly cloned into pAdTrack-CMV.

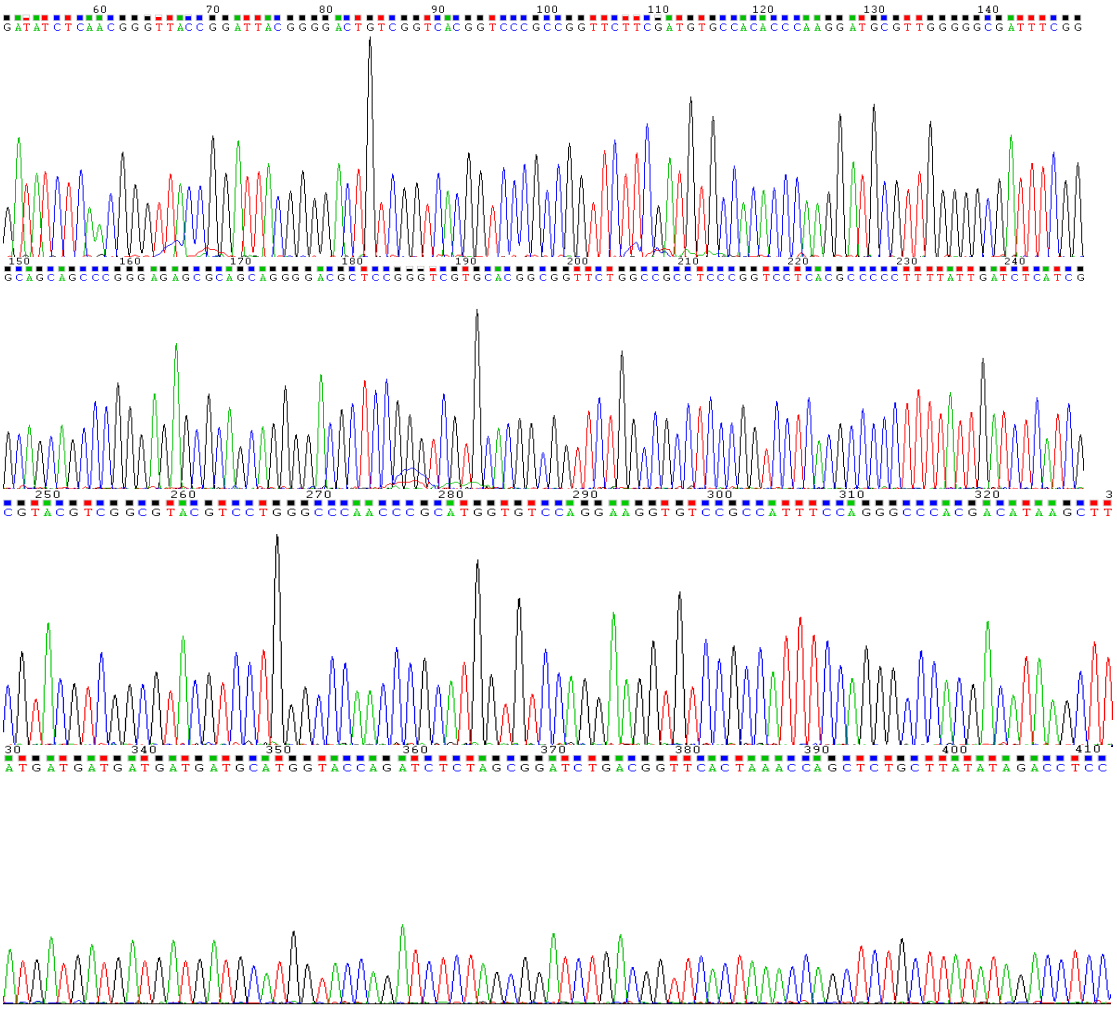

Fig.3. The antisense-sequencing identification of pAdTrack-H-ICP47

Identification of recombinant adenovirus vector pAdEasy-H-ICP47

The recombinant shuttle plasmid pAdTrack-H-ICP47 and the bone structure pAdeasy-1 were homologously recombined into BJ5183 cells. There were two bands at approximately 30 kb and 4.5 kb which were corresponded with the expectation after pAdTrack-H-ICP47 was digested with *Pac I*. The result of recombinant adenoviral vector pAdEasy-H-ICP47 identified by digestion of *Pac I* was shown in Fig. 4.

Fig.4. Identification of pAdEasy-H-ICP47 by endonuclease digestion with *Pac I*

Lane l：pAdEasy-H-ICP47；Lane 2：pAdEasy-H-ICP47/ *Pac I*； Lane 3： DNA Marker DL15000

Fluorescence photomicrograph observation

The verified pAd Easy-H-ICP47 was linearized with *Pac* Ⅰ and then transfected into 293 cells to generate adenovirus. Seven-ten days later, the adherent cells rounded and ablated. The green fluorescence could be seen under a fluorescence microscope (Fig. 5). High fluorescence intensity potentially correlated with high expression of His-tag-ICP47 fusion protein in 293 cells. The results of fluorescence photomicrograph observation verified that the recombinant adenoviruses of r-H-ICP47 and r-Track were successfully constructed and transfected into 293 cells. The viruses were amplified and the virus particle titres of r-H-ICP47 and r-Track were determined with the resulting of 3.7×10^{10}efu/mL and 4.4×10^{10}efu/mL.

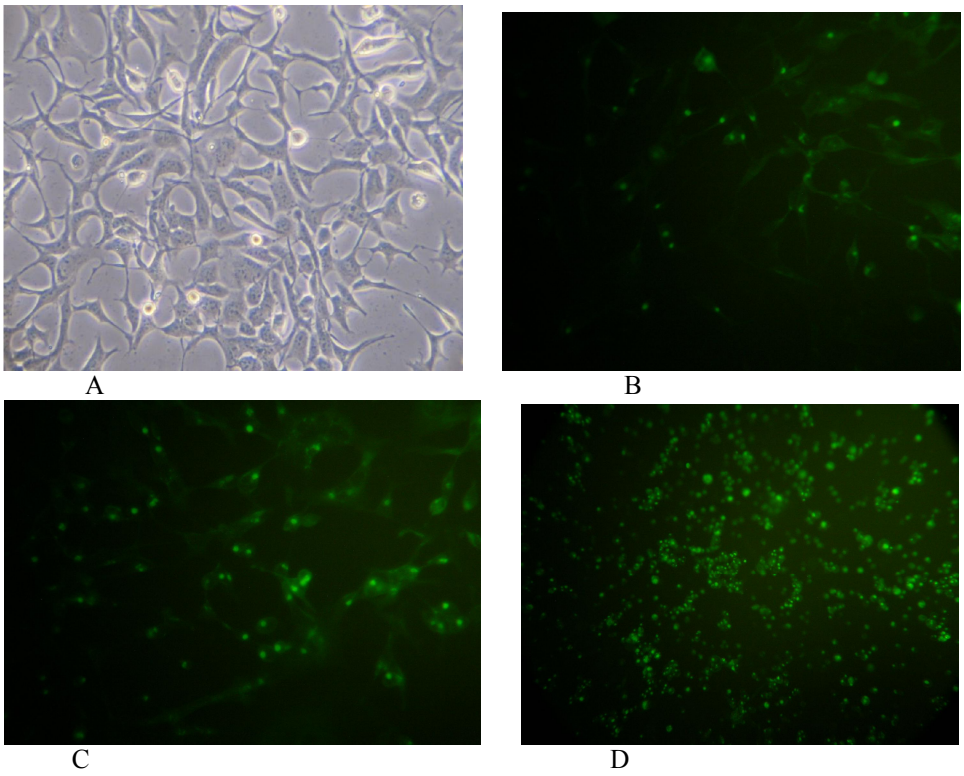

Fig.5 Transfection of recombinant adenovirus pAdEasy-H-ICP47 in 293 cells

A. Normal 293 cells before transfection of recombinant adenovirus（200×）; B Transfection of recombinant adenovirus cell at the 7th d（200×）; C Transfection of recombinant adenovirus at the 8th d（200×）; D Transfection of recombinant adenovirus at the 10th d（100×）.

PCR analysis of the expression of His-tag-ICP47 fusion gene

The result of PCR analysis showed that one band was detected at approximately 304bp in pAdEasy-H-ICP47 and pAdTrack-H-ICP47 samples and the band was in the right range consistent with the expected length, but no band was detected at approximately 304bp in the control empty adenovirus r-Track sample. The result of PCR analysis was shown in Fig. 6.

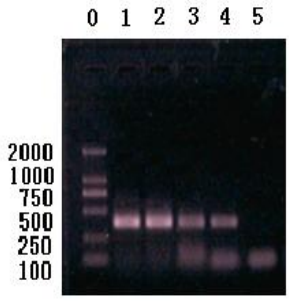

Fig. 6. Analysis of PCR amplification assay

Lane 0：DNA Marker DL2000；Lane 1：pAdEasy-H-ICP47；Lane 2：pAdEasy-H-ICP47；Lane 3：r-H-ICP47；Lane 4：r-H-ICP47；Lane 5：r-Track.

Western blot analysis of recombinant adenovirus -infected 293 cells

To confirm the proteins producted by r-H-ICP47-infected, r-Track-infected or mock-infected 293 cells, extracts were reacted with three kinds of antibodies in Western blot analysis (Fig. 7). The blots probed with anti-β-tubulin antibody were recognized in all cells extracts at approximately the same molecular mass (55 kDa). When an blot was probed with anti-GFP antibody, bands of extracts of r-H-ICP47-infected and r-Track-infected 293 cells were detected at approximately the same molecular mass (27 kDa), but no bands was detected in the mock-infected 293 cells. When an identical blot was probed with anti-6×His antibody, band of His-tag-ICP47 fusion protein was detected in extracts of r-H-ICP47-infected 293 cells (11kDa), but no band was detected in r-Track-infected and mock-infected 293 cells.

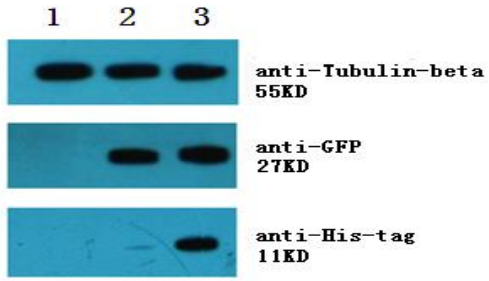

Fig. 7 Analysis of Western-blot assay
Lane1: mock-infected 293 cells; Lane 2: r-Track-infected 293 cells; Lane 3: r-H-ICP47-infected 293 cells

DISCUSSION

Adenovirus vectors offer many advantages, including high titer, broad range of infectivity, efficient gene transfer, limited pathological potential, and feasibility for delivery in vivo compared to plasmid vector, as has been reviewed previously[1,10]. They have become versatile tools for gene delivery and expression in the clinical trials and they have been used extensively in early clinical trials aimed at treatment of genetic disease[11] and cancer[12].

However, the response of host immune systems against foreign gene products expressed by genetically modified cells and/or vector-encoded proteins is a major obstacle to successful gene therapy. For these reasons, transient expression of the transgene has been reported in the majority of immunocompetent animal models, which is associated with problems with repeat administration of the vector and vector-induced inflammatory response [2].

Long-term persistence of gene-modified cells could potentially be achieved by using immunosuppressive regimens, which are commonly used in preventing of autoimmune disorders, solid organ graft rejection and graft-versus-host disease [13]. However, immunosuppressive regimens are not always effective and associated with the risk of infectious complications, and other regimen-related toxicities[3-4].

Survival of vertebrates is strongly dependent on the adaptive immune system and prevention of host immune response against transplanted cells and products of introduced genes could be a critical subject for the long-term success of gene therapy [14]. Inhibition of costimulatory interactions between T cells and antigen- presenting cells, such as blocking monoclonal antibody (MAb) against CD40 ligand and CD28-CD40 signaling with CTLA4Ig, has been reported in many studies of adenovirus-mediated gene therapy [15-17]. However, cellular and humoral immune responses were markedly reduced but not completely abrogated in these studies. On the cellular level, MHC class I–restricted pathway of antigen processing is critical for elimination of most virus infections, tumor surveillance, transplantation rejection, graft-versus-host reactions, and has been an attractive target for immune rejection. Blocking MHC Ⅰ antigen expression is becoming a research hotspot of inducting immune tolerance[6], and efficient antigen presentation restricted by MHC class I is associated with TAP[18]. As a member of the ATP binding cassette protein family, TAP plays a critical role in transporting cytosolic peptides across the membrane of endoplasmic reticulum (ER) for assembly with MHC class I heavy chain and β_2-microglobulin (β_2m). Endogenous peptide presentation is affected by inhibition of TAP[19-20]. In the absence of functional TAP and antigenic peptides, most MHC class I molecules are eventually redirected to the cytosol and degraded by proteasomes [21-24].

ICP47, which is an immediate-early protein expressed by HSV-1 and an 88–amino acid cytosolic polypeptide, binds to the TAP1–TAP2 heterodimer in human but not in mouse cells and inhibits transport of proteasomally generated antigenic peptides from mostly cytosolic proteins into the endoplasmic reticulum (ER), where they would be loaded onto freshly synthesized HLA class I molecules. As a consequence, MHC class I molecules fail to be loaded with peptides, and the empty MHC class I molecules are retained in the ER and presentation of epitopes to $CD8^+$ T cells is abolished in HSV-infected human cells [7-8].Thereby the HSV-infected human cells are not lysed by cytotoxic T lymphocytes (CTL) and effectively evade the immune response in humans just as during HSV infection in vivo[25-27].

To investigate further the role of ICP47 in the elimination of transgene expression, we constructed an adenovirus vector expressing the His-tag-ICP47 fusion protein. In our study, AdEasy-1 system shuttle plasmid

contains a GFP gene incorporated into the adenoviral backbone, allowing direct observation of the efficiency of transfection and infection, which is more convenient for operation[28], and 6×His tag also facilitates detection by using biotinylated anti-6×His antibody and enables purification and detection of recombinant adenovirus without affecting tropism or production[29]. Furthermore the His-tag-ICP47 fusion gene in the E1/E3-deleted construct which is under the control of the cytomegalovirus promoter could block the expression of other viral proteins and minimizing unwanted effects on antigen processing.

In conclusion, a recombinant vector expressing the His-tag-ICP47 fusion protein is successfully constructed and the proteins producted by r-H-ICP47-infected cells are confirmed by PCR and Western blot analysis. In the following studies, we should verified the immunological activities of the His-tag-ICP47 fusion protein by cells and animal experiments and expect those findings should have important implications for analyzing the mechanisms of immune tolerance as well as human gene therapy. Moreover, we expect these studies should open up new horizons for expanding the fields of viral immunology, exploring the interactions between host immune systems and viruses, and enable us to explore more effective preventions and treatments for clinical diseases.

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References

[1] AC Silva, C Peixoto, T Lucas, C Kuppers, PE Cruz, PM Alves, and S Kochanek. Adenovirus vector production and purification [J]. Curr Gene Ther, 2010, 10(6): 437-455.

[2] Peter Johannes Holst, Cathrine Ørskov, Allan Randrup Thomsen, et al. Quality of the Transgene-Specific $CD8^+$ T Cell Response Induced by Adenoviral Vector Immunization Is Critically Influenced by Virus Dose and Route of Vaccination [J]. J. Immunol., 2010, 184 (8): 4431- 4439.

[3]U Christians, V Schmitz, W Schoning, J Bendrick-Peart, J Klawitter, M Haschke, and J Klawitter. oxicodynamic therapeutic drug monitoring of immunosuppressants: promises, reality, and challenges [J]. Ther Drug Monit, 2008, 30(2): 151-158.

[4] Albert MH, Anasetti C, Yu XZ. Cozza. T regulatory cells as an immunotherapy for transplantation [J]. Expert Opin Biol Ther. 2006, 6(4):315-324.

[5]Christopher Aisenbrey, Christina Sizun, Joachim Koch, Meike Herget, Rupert Abel, Burkhard Bechinger,and Robert Tampe. Structure and Dynamics of Membrane-associated ICP47,a Viral Inhibitor of the MHC I Antigen-processing Machinery [J]. J. Biol. Chem., 2006; 281(41): 30365 - 30372.

[6]Nathan J. Felix, W. June Brickey, Robert Griffiths, et al. H2-DM$\alpha^{-/-}$- Mice Show the Importance of Major Histocompatibility Complex–bound Peptide in Cardiac Allograft Rejection [J]. J. Exp., 2000.192(1):192: 31-40

[7] Margit H. Lampen, Marieke C. Verweij, Bianca Querido, et al. $CD8^+$ T Cell Responses against TAP-Inhibited Cells Are Readily Detected in the Human Population [J]. J. Immunol., 2010, 185(11): 6508 -6517.

[8] Christopher Aisenbrey, Christina Sizun, Joachim Koch, et al. Structure and Dynamics of Membrane-associated ICP47, a Viral Inhibitor of the MHC I Antigen-processing Machinery [J]. J. Biol. Chem., 2006, 281 (41): 30365-30372.

[9] Huang-Ge Zhang, Jinfu Xie, Igor Dmitriev, Elena Kashentseva, David T. Curiel, Hui-Chen Hsu, and John D. Mountz. Addition of Six-His-Tagged Peptide to the C Terminus of Adeno- Associated Virus VP3 Does Not Affect Viral Tropism or Production [J]. J VIROL, 2002, 76(23):12023–12031.

[10] Raviprakash K, Wang D, Ewing D, Holman DH, Block K, Woraratanadharm J, Chen L, Hayes C, Dong JY, Porter K. A tetravalent dengue vaccine based on a complex adenovirus vector provides significant protection in rhesus monkeys against all four serotypes of dengue virus. [J]. J Virol. 2008, 82 (14):6927-6934.

[11] Eto Y, Yoshioka Y, Asavatanabodee R, Mizuguchi H, Mukai Y, Okada N, Nakagawa S. Development of pegylated adenovirus vector for cancer gene therapy. [J]. Yakugaku Zasshi. 2008, 128(12):1733-1742.

[12]Furukawa K, Ohashi T, Haruki K, Fujiwara Y, Iida T, Shiba H, Uwagawa T, Kobayashi H, Yanaga K. Combination treatment using adenovirus vector-mediated tumor necrosis factor-alpha gene transfer and a NF-κB inhibitor for pancreatic cancer in mice [J]. Cancer Lett. 2011, 306(1):92-98.

[13]Liu QF, Fan ZP, Zhang Y, Jiang ZJ, Wang CY, Xu D, Sun J, Xiao Y, Tan H. Sequential intensified conditioning and tapering of prophylactic immunosuppressants for graft-versus-host disease in allogeneic hematopoietic stem cell transplantation for

refractory leukemia [J]. Biol Blood Marrow Transplant. 2009, 15(11):1376-1385.
[14]Chaanine AH, Kalman J, Hajjar RJ. Cardiac gene therapy [J]. Semin Thorac Cardiovasc Surg. 2010, 22(2):127-139.
[15]Xiao B, Xia W, Zhao K, Yang Y, Fu S, Li Y, Liu B, Guo S. Ex vivo transfer of adenovirus- mediated CTLA4Ig gene combined with a short course of rapamycin therapy prolongs free flap allograft survival [J]. Plast Reconstr Surg. 2011, 127(5):1820-1829.
[16] Iida T, Shiba H, Misawa T, Ohashi T, Eto Y, Yanaga K. Immunogene therapy against colon cancer metastasis using an adenovirus vector expressing CD40 ligand [J]. Surgery. 2010, 148(5):925-935.
[17] Wang X, Hao J, Metzger DL, Mui A, Ao Z, Verchere CB, Chen L, Ou D, Warnock GL. Local expression of B7-H4 by recombinant adenovirus transduction in mouse islets prolongs allograft survival[J]. Transplantation. 2009, 27;87(4):482-490.
[18]L Lybarger, X Wang, M Harris, et al. Viral immune evasion molecules attack the ER peptide- loading complex and exploit ER-associated degradation pathways [J]. Curr Opin Immunol, 2005, 17 (1): 71-78.
[19]J Koch, R Tampe. The macromolecular peptide-loading complex in MHC class I-dependent antigen presentation [J]. Cell Mol Life Sci, 2006, 63 (6): 653-662.
[20]T Elliott, A Williams. The optimization of peptide cargo bound to MHC class I molecules by the peptide-loading complex [J]. Immunol Rev, 2005, 207 (10): 89-99.
[21]Maaike E. Ressing, Sinéad E. Keating, Daphne van Leeuwen, et al. Impaired Transporter Associated with Antigen Processing-Dependent Peptide Transport during Productive EBV Infection [J]. J. Immunol., 2005, 174 (11): 6829-6838.
[22]Danijela Koppers-Lalic, Eric A. J. Reits, Maaike E. Ressing, et al. Varicelloviruses avoid T cell recognition by UL49.5-mediated inactivation of the transporter associated with antigen processing [J]. PNAS, 2005, 102 (14): 5144- 5149.
[23]N Nozawa, N Inoue. Mechanisms of congenital CMV infection [J]. Nippon Rinsho, 2006, 64 (Suppl 3): 446-450
[24]Nupur T. Pande, Colin Powers, Kwangseog Ahn, et al. Rhesus Cytomegalovirus Contains Functional Homologues of US2, US3, US6, and US11 [J].J. Virol., 2005, 79 (9): 5786 - 5798.
[25]M Hong, WZ Li, QH Li. The immune escape mechanism of herpes simplex virus type 1 suppression of transporter associated with antigen processing (TAP) by ICP47 [J]. Bing Du Xue Bao, 2007, 23 (1): 72-75.
[26]Liesbeth E. M. Oosten, Danijela Koppers-Lalic, Els Blokland, et al. TAP-inhibiting proteins US6, ICP47 and UL49.5 differentially affect minor and major histocompatibility antigen-specific recognition by cytotoxic T lymphocytes [J]. Int. Immunol., 2007, 19 (9): 1115 - 1122.
[27]Christopher Aisenbrey, Christina Sizun, Joachim Koch, et al. Structure and Dynamics of Membrane-associated ICP47, a Viral Inhibitor of the MHC I Antigen-processing Machinery [J]. J. Biol. Chem., 2006, 281 (41): 30365 - 30372.
[28]Zhang XW, Meng ZH, Zhao JS, Zhang D, Xie YJ, Gao SH, Wang CY. Gene therapy targeting for carcinoma regulated by E2F-1 promoter [J]. Zhonghua Wai Ke Za Zhi. 2006, 44(23):1636- 1639.
[29]Wu Q, Fu Q, Chen Q, Cai Q, Fan Z, Zhan Z, Niu L, Pei H, Zhou P. Prokaryotic expression, identification and bioinformatics analysis of fbpB-esxA fusing gene from Mycobacterium tuberculosis [J]. Asian Pac J Trop Med. 2011, 4(7):530-534.

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