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Successful Expression of DNA-Based Vaccine Construct Encoding Human Papillomavirus Type 16 E7 Fused to Heat Shock Protein B1 in Eukaryotic Cells

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ABSTRACT

Introduction: Developing potent therapeutic vaccines against human papillomaviruses (HPVs) is crucial for the effective management of various HPV-associated cancers. DNA-based vaccines are attractive due to their safety, stability, and capacity to elicit a targeted immune response against specific antigens. Heat shock proteins (HSPs) can enhance the efficacy of DNA vaccines when used as adjuvants. In this study, we created a recombinant DNA molecule by fusing the *HPV16 e7* gene with either the *hspB1* or *hsp27* gene and assessed its expression in a eukaryotic cell line. **Methods:** Initially, we constructed a recombinant eukaryotic expression vector by inserting the *hsp27-e7* fusion gene into the pcDNA3.1 (-) vector. The concentration and purity of the sample were evaluated using NanoDrop spectrophotometry. We cultured human embryonic kidney 293T (HEK-293T) cells in RPMI 1640 medium and transfected them with the pcDNA3.1-*hsp27-e7* construct using Lipofectamine 2000 transfection reagent. After 48 hours, we assessed the expression of the Hsp27-E7 fusion protein by western blotting using an anti-E7 monoclonal antibody. **Results:** We successfully subcloned the *hsp27-e7* fusion gene into the pcDNA3.1 (-) vector, and enzymatic digestion confirmed a distinct ~975 bp band on an agarose gel. The concentration and purity of the recombinant DNA vector in a 10 mL culture were measured to be 210 ng/µL and 1.86, respectively. Furthermore, the expression of the Hsp27-E7 fusion protein in HEK-293T cells was confirmed by Western blot analysis, which detected a distinct band of approximately 38 kDa. **Conclusion:** Our *in vitro* findings demonstrate successful expression of the DNA construct encoding the *hsp27-e7* gene, which can be utilized as a DNA vaccine for future *in vivo* investigations.

INTRODUCTION

Approximately 15 high-risk HPV types are strongly associated with developing genital cancers [1]. Among the high-risk HPVs associated with genital cancers, HPV 16 is the most prevalent oncogenic type [2]. The HPV genome encodes eight proteins that can be classified into two groups: six early (or regulatory) proteins (E1, E2, E4, E5, E6, and E7) and two late (or capsid) proteins (L1 and L2). The early proteins of HPV play a crucial role in genome replication and immune modulation, whereas the late proteins primarily facilitate virus transmission [1]. Currently, there are three licensed prophylactic vaccines available to prevent high-risk HPV infection: Gardasil, Cervarix, and Gardasil-9. In contrast, therapeutic vaccines have been developed to eliminate HPV-related cancers by inducing cell-mediated immunity rather than relying on neutralizing antibodies [3]. There are currently no officially approved therapeutic vaccines available for HPV-related cancers [3, 4]. Several vaccination strategies have advanced to phase III clinical trials, including a bacterial vector vaccine called ADXS11-001 and a DNA vaccine called VGX3100. VGX-3100 (NCT01304524) is a DNA vaccine that targets the E6/E7 antigens of HPV16/18 and utilizes electroporation as a delivery method [3]. The *E6* and *E7* genes are considered the most promising targets for therapeutic vaccination against HPV-related cancers [3, 4].

Among the available therapeutic vaccines, DNA vaccines are considered safe, cost-effective, and stable and can be quickly produced with high purity [5]. Nevertheless, weak immunogenicity and low delivery

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remain significant challenges that can be overcome using adjuvants and delivery systems. Recently, heat shock proteins (HSPs) have been increasingly utilized as antigens or adjuvants in various vaccine strategies for cancer and infectious diseases [6]. HSPs are classified into six families based on their molecular weights: HSP90, HSP70, HSP60, HSP40, HSP100, and small HSPs [7]. Small HSPs, which contain a conserved α-crystallin domain, are highly conserved across species. Among them, the multifunctional protein HspB1 (also known as Hsp27) is expressed in all human tissues. HspB1 plays a critical role in protein chaperoning, antioxidant pathways, and suppressing apoptosis. It also has potential applications as a biomarker for infection diagnosis and as a therapeutic target in cancer treatment [8].

The main objective of this study was to construct a DNA vaccine by linking the HPV16 *e7* gene (K02718) with the *Mus musculus hsp27* gene (NM_013560) and to evaluate its expression in mammalian cells using the Lipofectamine transfection reagent.

MATERIAL AND METHODS

Generation of a recombinant pcDNA3.1-*hsp27-e7* plasmid. Our group previously synthesized the *hsp27-e7* fusion gene using an expression vector (pEGFP-N3, Biomatik Co., Canada). To generate the pcDNA3.1-*hsp27-e7* plasmid (shown in Figure 1), we digested pEGFP-*hsp27-e7* and pcDNA3.1 (Invitrogen) using *Nhe*I/*Bam*HI restriction enzymes (Fermentas, Germany). The *hsp27-e7* fragment was then ligated to pcDNA3.1 (-) using T4 DNA ligase (Fermentas, Germany). The pcDNA3.1-*hsp27-e7* plasmid was then transformed into *Escherichia coli* DH5α competent cells using a heat shock method, followed by a 90-second incubation at 42 °C. A single clone was cultured overnight in Luria-Bertani (LB) broth at 37 °C. The recombinant plasmid was purified using a plasmid DNA extraction mini-kit (Yekta Tajhiz Azma, Iran) and subsequently confirmed through digestion analysis. The concentration and purity of the purified plasmid DNA were determined using NanoDrop spectrophotometry.

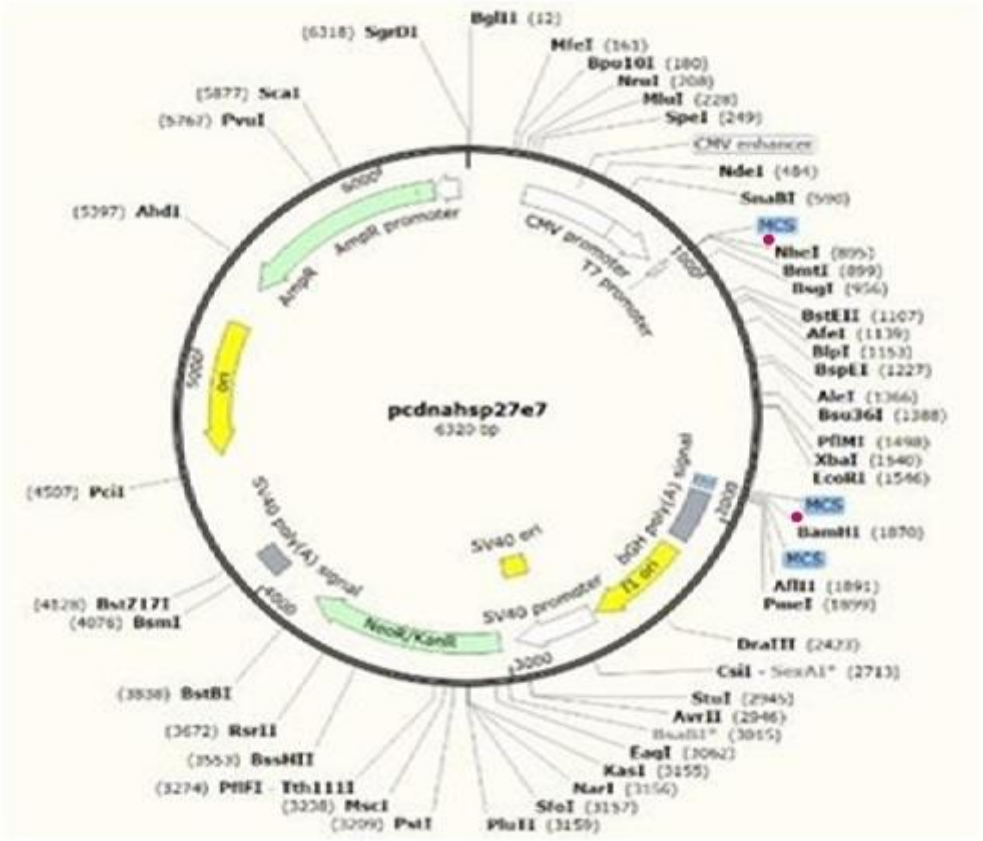


Fig. 1. The pcDNA3.1-*hsp27-e7* construct was designed by SnapGene software. The gene cloning site is denoted by the red dot in the construct

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Cell culture. Human embryonic kidney 293T (HEK-293T) cells obtained from the Pasteur Institute of Iran were cultured in RPMI 1640 medium (Sigma, Germany) supplemented with 10% fetal bovine serum (Gibco, Germany). A total of 5×10^4 cells were counted and seeded into 24-well plates 24 h prior to transfection.

Transfection. HEK-293T cells at 70%-80% confluency were transfected with either pcDNA3.1-*hsp27-e7* or the empty vector (pcDNA3.1) using Lipofectamine 2000 reagent (Sigma, Germany). To form lipofectamine-plasmid DNA complexes, 2 µl of lipofectamine was mixed with 50 µl of serum-free medium and incubated at room temperature for 5 min. Next, 1 µg of plasmids was mixed with 50 µl of serum-free medium and added to the lipofectamine solution. The combination was incubated at room temperature for 20 min. The transfection complexes were added to each well, and after 6 h of incubation at 37 °C, the culture medium was replaced with RPMI 10%. The transfection efficiency was evaluated by Western blotting 48 h after transfection.

Western blotting. The cells were harvested using trypsin and subsequently resuspended in PBS. Total cellular proteins were extracted by mixing them with 2X sample buffer (a mixture of Tris-HCl, glycerol, SDS, 2-mercaptoethanol, and bromophenol blue; Sigma, Germany), separating them by 12.5% SDS-PAGE, and transferred onto a nitrocellulose membrane. The membrane was blocked with a mixture of TBS 10X, 0.1 % Tween 20, and bovine serum albumin (Sigma, Germany) and then it was washed with a solution of TBS 10X and 0.1% Tween 20 (Sigma, Germany). The membrane was incubated with an anti-E7 monoclonal antibody (Abcam, USA) diluted at a ratio of 1:5000 (v/v). The Hsp27-E7 fusion protein was detected using an anti-mouse horseradish peroxidase (HRP)-conjugated antibody (diluted 1:10000; Sigma, Germany) in the presence of 3,3'-diaminobenzidine (DAB) substrate.

RESULTS

Confirmation of recombinant pcDNA3.1-*hsp27-e7* plasmid. Confirmation of recombinant pcDNA3.1-*hsp27-e7* plasmid expression was achieved by observing a ~975 bp band for the *hsp27-e7* fusion gene and ~5427 bp for the pcDNA3.1 (-) plasmid after digestion with *Nhe*I/*Bam*HI restriction enzymes (Fig. 2). The concentration and purity of the recombinant DNA plasmid were determined to be 210 ng/µL (for a 10 mL of culture) and 1.86, respectively.

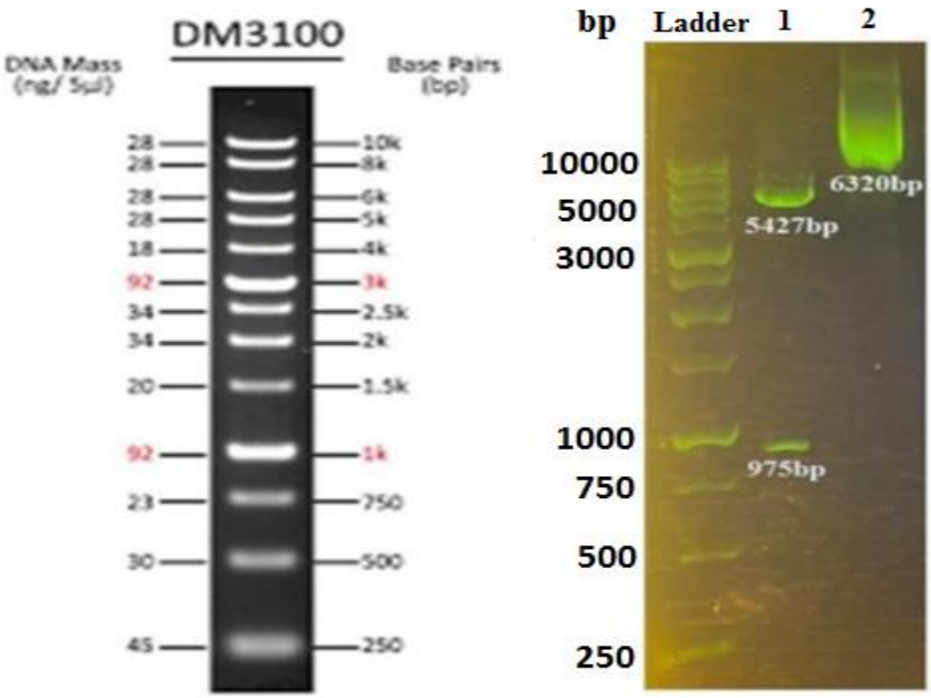


Fig. 2. Confirmation of recombinant plasmid pcDNA3.1-*hsp27-e7* by *Nhe*I/*Bam*HI digestion and agarose gel electrophoresis. Lane 1 shows the ~975 bp digested product of the plasmid, while Lane 2 displays the purified plasmid on a 1% agarose gel. Ladder represents the DNA size (bp; Fermentas, Germany).

Hsp27-E7 protein expression in HEK-293T cells. Successful expression of the Hsp27-E7 fusion protein was confirmed by western blot analysis using an anti-E7 monoclonal antibody. Western blot analysis revealed a distinct band at approximately 38 kDa, corresponding to the Hsp27-E7 fusion protein (Fig. 3).

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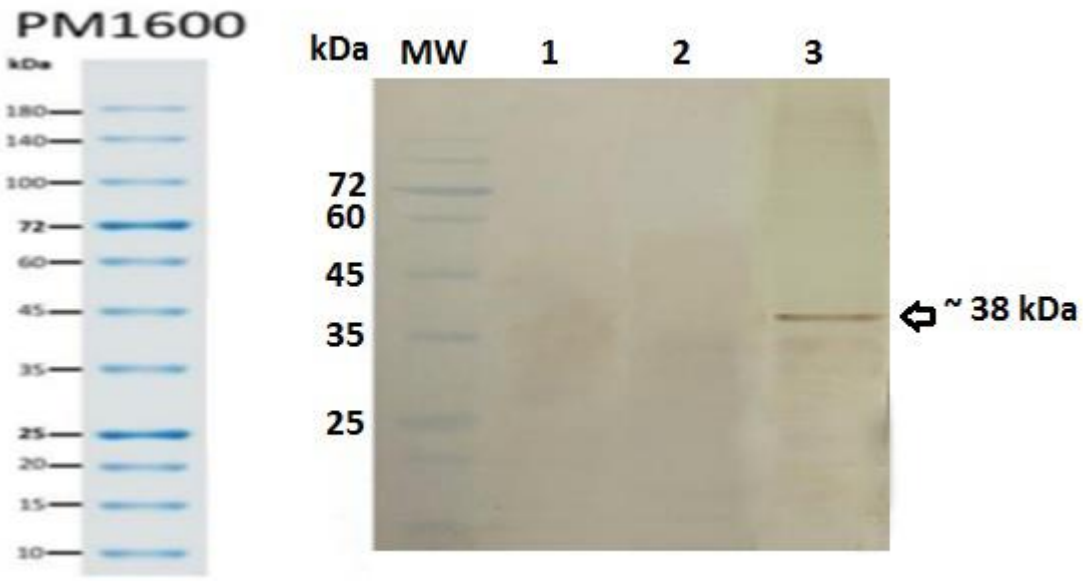


Fig. 3. Western blot analysis of Hsp27-E7 fusion protein expression in HEK-293T cells using an anti-E7 monoclonal antibody. Lane 1: non-transfected cells; Lane 2: cells transfected with empty vector (pcDNA3.1); Lane 3: cells transfected with pcDNA3.1-*hsp27-e7*, expressing ~38 kDa Hsp27-E7 protein. MW represents the protein molecular weight marker (kDa, Fermentas, Germany).

DISCUSSION

Cervical cancer is a prevalent cancer with a high global mortality rate among women. Human papillomaviruses (HPVs) are the primary causative agents of cervical cancer, and therapeutic vaccines have been developed to induce T-cell-mediated immunity and eradicate the virus. Therapeutic HPV vaccines primarily target antigens based on HPV 16/18 E6 and/or E7 proteins. Therapeutic DNA vaccines have demonstrated safety, cost-effectiveness, and stability, positioning them as promising candidates for further advancement into phase III clinical trials [9, 10]. Several methods have been explored to enhance the efficacy of DNA vaccines, including novel DNA vector design and the utilization of adjuvants or delivery systems [11]. Adjuvants can enhance the effectiveness of vaccination strategies by promoting an appropriate immune response against infections [8]. Heat shock proteins have been identified as effective adjuvants in vaccine development [12, 13]. For example, HspB1 (Hsp27) has been demonstrated as an effective adjuvant for increasing antigen-specific immune responses in developing therapeutic HIV-1 vaccines [14].

This study aimed to assess the expression of the Hsp27-E7 fusion protein in a human cell line via western blot analysis. To achieve this objective, the *hsp27-e7* fusion gene (~975 bp) was subcloned into a eukaryotic expression vector, designated as pcDNA3.1-*hsp27-e7*. Next, HEK-293T cells were transfected with purified pcDNA3.1-*hsp27-e7* using Lipofectamine 2000 reagent. Transfection methods can be broadly categorized into three main groups: biological, chemical, and physical delivery systems [15, 16]. In this study, a cationic lipid (Lipofectamine) was used for DNA delivery. Cationic lipids have emerged as promising non-viral gene delivery vehicles that can preserve the biological activity of genes and protect them from degradation in lysosomes [13]. For example, Zhou *et al*. (2017) utilized Lipofectamine 2000 to deliver pEGFP-*sag4* to HEK-293T cells as a component of their DNA vaccine development strategy against *Toxoplasma gondii* infection [17]. Furthermore, mammalian expression cells can produce recombinant proteins with proper folding and biological activity. Various mammalian cell lines, including human embryonic kidney (HEK) and Chinese hamster ovary (CHO), have been utilized for protein expression. HEK-293T cells have been widely used to produce recombinant proteins through both transient and stable transfection methods [18]. Therefore, we used the HEK-293T cell line for protein expression. The most commonly used method for protein detection is Western blotting, which identifies proteins based on their specific binding to antibodies. In this study, Western blotting was conducted using an anti-E7 monoclonal antibody to confirm the proper expression of the Hsp27-E7 protein. Cells transfected with pcDNA3.1-*hsp27-e7* expressed an expected band of approximately 38 kDa, whereas cells transfected with empty pcDNA3.1 did not exhibit this band.

In summary, we achieved the *in-vitro* expression of the *hsp27-e7* fusion gene by delivering the recombinant pcDNA3.1-*hsp27-e7* into HEK-293T cells using lipofectamine. Considering its demonstrated appropriate expression, this DNA fusion construct will be utilized as a potential therapeutic DNA vaccine candidate against HPV for *in vivo* investigations.

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CONFLICT OF INTEREST

The authors declare no that there are conflicts of interest associated with this manuscript.

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