Short article

Design and Construction of a Cloning Vector Containing the *hspX* Gene of *Mycobacterium tuberculosis*

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Abstract

Background: Tuberculosis (TB) is a major cause of death worldwide. Finding an effective vaccine against TB is the best way to control it. Several vaccines against this disease have been developed but none are completely protective. The aim of this study was to design and construct a cloning vector containing the *Mycobacterium tuberculosis* (*M. tuberculosis*) heat shock protein X (*hspX*).
Methods: First, an *hspX* fragment was amplified by PCR and cloned into plasmid pcDNA3.1(+) and recombinant vector was confirmed.
Results: A 435 bp *hspX* fragment was isolated. The fragment was 100% homologous with *hspX* of M. tuberculosis strain H37Rv in GenBank.
Conclusions: In this study, the cloning vector pcDNA3.1(+), containing a 435-bp *hspX* fragment of *M. tuberculosis,* was constructed. This could be used as a DNA vaccine to induce immune responses in animal models in future studies.

Keywords: Cloning, DNA vaccine, *hspX*, *Mycobacterium tuberculosis*

Introduction

Mycobacterium (M.) tuberculosis was first identified as the cause of tuberculosis (TB) by Robert Koch in 1882. Tuberculosis (TB), for thousands of years, was known as the "human plague" (1). Tuberculosis is one of the most important infectious diseases and a major cause of death worldwide, especially in developing countries. One-third of the world's population is infected with *M. tuberculosis,* and every second another individual is added to them (2).

According to the World Health Organization (WHO), approximately 7.1 billion people are infected with *M. tuberculosis* and more than 20 million of those suffer from the disease. More than 9 million individuals are infected every year, resulting in about 1.5 million deaths. *M.*

tuberculosis infections, with malaria and AIDS, are considered to have the highest death rates of all infectious diseases (3-6).

Currently the Bacillus Calmette-Guerin (BCG) vaccine is the only TB vaccine in use in many countries. Although the BCG vaccine protects against TB in children, it is not effective against pulmonary TB in adults (6-8)

Heat shock protein X (*hspX*) has been purified from *M. tuberculosis*. Heat shock protein X has a proposed role in the maintenance of long-term viability during latent, asymptomatic infections, and in replication during initial infection (9, 10). This antigen stimulates CD4+ and CD8+ T cells and induces TNF-α, IL-2, and IFN-γ expression (11).

In the present study, *hspX* from *M. tuberculosis*

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was cloned into plasmid pcDNA3.1(+). This construct could be used as a DNA vaccine to induce immune responses in animal models in future studies.

Materials and Methods

1. PCR amplification of hspX

In this study, *M. tuberculosis* strain H37Rv (Pasteur institute of Iran) DNA was extracted by boiling as described previously (4,12). *hspX* was amplified by the polymerase chain reaction (PCR) using forward (5'tatttcggatccaccatggccaccacccttcccgtt3') and reverse (5'attatttctagattatcagttggtggaccggatctgaat3') primers. The PCR reaction was prepared and gene amplification was performed for 4 min at 95℃, followed by 35 cycles at 94℃ for 30 sec, 59℃ for 30 sec, and 72℃ for 30 sec. The final amplification was performed for 10 minutes at 72℃. PCR products were analyzed using 1.5% agarose gel and Green viewer staining (ParsTous, Iran).

2. Cloning of hspX into pcDNA3.1(+)

The amplified *hspX* fragment and pcDNA3.1(+) were digested with *Bam*HI and *Xba*I (Fermentas, Germany) restriction enzymes. Digested products were purified with a gel purification kit (Bioneer, South Korea) according to the manufacturer's recommendations. For insertion of *hspX* into pcDNA3.1(+), we used ligation solution containing 14.5 μl of digested and purified pcDNA3.1(+), 8 μl of digested and purified *hspX* DNA, 2.5 μl of *T4 DNA ligase* buffer, 0.2 μl of *T4 DNA ligase* (Vivantis, Malaysia), and 2 μl of polyethylene glycol (PEG) (Thermo Scientific, USA). The mixture was incubated overnight at 16℃ as described previously (12, 13). *Escherichia (E.) coli* strain TOP10 cells were transformed with this construct. Transformants were confirmed by colony-PCR using the hspX-specific primers described above, restriction enzyme digestion with *Bam*HI and *Xba*I, and sequencing (MacroGen, South Korea).
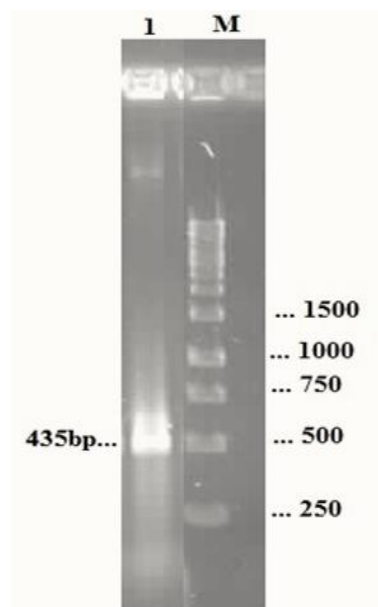
Results

In this study, an *hspX* fragment was amplified by PCR from *M. tuberculosis* H37Rv genomic DNA (Figure 1) and cloned into pcDNA3.1 (+). In colony-PCR a fragment of 435 bp was observed (Figure 2). Presence of the 435 bp fragment was confirmed by double-digestion

of the recombinant vector. DNA sequencing showed the fragment was 100% homologous with *hspX* of *M. tuberculosis* strain H37Rv recorded in GenBank.
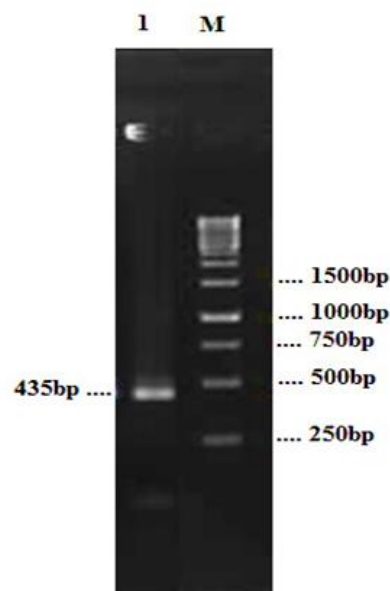
Fig .1. Agarose Gel Electrophoresis of *hspX* PCR Product. Lane 1: 435 bp *hspX* PCR product; lane M: 1 kb DNA size marker

Fig .2. Colony-PCR results using hspX-specific primers. Lane 1: PCR product of hspX, Lane M: 1 kb DNA size marker

Discussion

Tuberculosis is caused by infection with *M. tuberculosis* and a major cause of death worldwide. Currently, almost a third of the world's population is asymptomatically infected with *M. tuberculosis* (14,15). The increased prevalence of TB depends on three main factors: the AIDS pandemic, the emergence of multidrug-resistant TB strains, and the failure of vaccination programs (6, 16).

Although BCG as a vaccine against TB was approved, it cannot prevent latent TB infections. To control this disease it will be necessary to design a vaccine that can enhance immunogenicity (6, 12). Recently, many studies have attempted to produce effective vaccines against TB. These vaccines include viral vectors, DNA vaccines, subunit vaccines with fusion proteins, and recombinant BCG (9, 17, 18).

DNA vaccines can stimulate both humoral and cellular immunity. This property can be useful to prevent infections caused by intracellular pathogens, such as *M. tuberculosis* (4, 19). Several antigens, including Ag85B, TB10.4, AG85BAg85B, ESAT-6, MPB83, MPB32, and *hspX* HSPX are TB vaccine candidates (20, 21).

Shi et al. studied T cell responses against the *M. tuberculosis Ag85B* and *hspX* gene products and suggested that latency antigens of *M. tuberculosis* may be promising targets for developing more effective recombinant BCG strains than are presently available to protect against TB (22).

In an effort by Niu et al. to find an effective vaccine to increase the immunogenicity and protective efficacy of BCG, and control or eliminate *M. tuberculosis* in all stages of infection, antigens of MTB10.4, expressed in replicating bacilli, and *hspX,* expressed in dormant bacilli, were fused to produce a subunit vaccine. IFN-γ and IL-17 expression was greater in spleen lymphocytes of mice vaccinated with the subunit vaccine than in those of the control group, who were vaccinated with BCG. These findings suggest that HSPX-MTB10.4 has the potential to be a good multistage TB vaccine candidate (23).

Taylor et al. demonstrated that HSPX has the potential to elicit both short- and long-term protective effects. Recent studies have shown that IFN-γ production in cells from mice vaccinated with HSPX is significantly greater than that of mice vaccinated with BCG. The ability of HSPX to stimulate CD4+ T cells was also evaluated. For this purpose, spleen cells from mice were examined six months after the vaccination. The number of CD4+ T cells and abundance of cytokines TNF-α, IL-2, and IFN-γ in the cells of mice vaccinated with *hspX* were greater than those of mice vaccinated with BCG. Thus, HSPX may be a good TB vaccine candidate (11).

Haldar et al. analyzed *GlcB* and *hspX* DNA and proteins for early detection and treatment of tuberculosis meningitis (TBM) in children. Cerebral spinal fluid samples from 555 TBM-positive children (*M. tuberculosis*-culture positive, n = 29) were collected from three centers in Delhi. In this test, TBM (true positives) and NTIM (true negative) groups were defined and samples from both groups were analyzed by PCR and ELISA and compared for sensitivity. The ELISA showed 100% sensitivity and 96-97% specificity in the TBM samples. This study showed that GlcD and hspX can be appropriate targets for early diagnosis of TBM and accelerating the detection process (24).

In another effort to develop an effective DNA vaccine against *M. tuberculosis*, the mycobacterial genes, *Rv3407*, *Ag85A*, and *hspX* were joined in a single open reading frame by 2A sequences and the resulting recombinant subunit vaccine was called V-2A. V-2A injected into mice elicited both cellular and humoral immune responses against all three antigens. This vaccine also protected the mice against *M. tuberculosis* aerosol challenge. This result can help in the design and development of efficacious recombinant subunit vaccines in future studies (25).

In this study, *hspX* of *M. tuberculosis* H37Rv was amplified by PCR and cloned into pcDNA3.1(+). The construct may be used as a DNA vaccine to induce immune system responses in animal models in future studies.

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