Reports of Biochemistry & Molecular Biology
Vol.5, No.1, Oct 2016

Short article www.RBMB.net

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

Atieh Yaghoubi[1], Ehsan Aryan[1], Hosna Zare[1], Shadi Alami[1], Roghayeh Teimourpour[2], Zahra Meshkat*[1]

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*

1: Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
2: Department of Microbiology, School of Medicine, Ardabil University of Medical Sciences, Ardabil, Iran.
***Corresponding authors: Zahra Meshkat; Tel: +98 51 38012453; Fax: +98 51 38002960; E-mail: meshkatz@mums.ac.ir**
Received: Feb 10, 2016; Accepted: Apr 10, 2016

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°C, followed by 35 cycles at 94°C for 30 sec, 59°C for 30 sec, and 72°C for 30 sec. The final amplification was performed for 10 minutes at 72°C. 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°C 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.

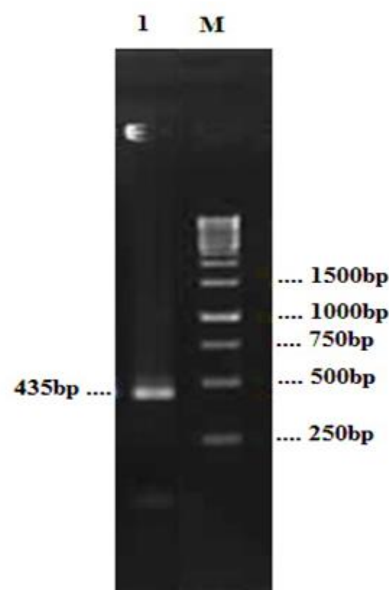

Fig .1. Agarose Gel Electrophoresis of *hspX* PCR Product. Lane 1: 435 bp *hspX* PCR product; lane M: 1 kb DNA size marker

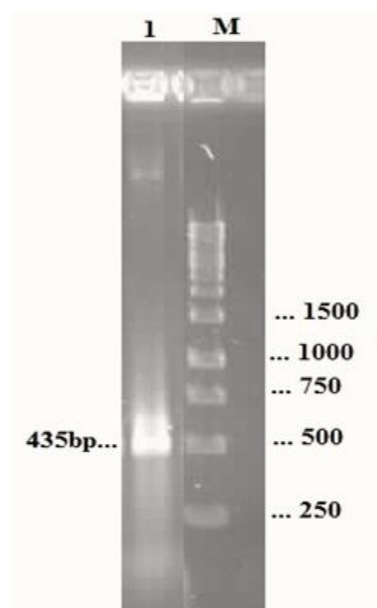

Fig .2. Colony-PCR results using hspX-specific primers. Lane 1: PCR product of hspX, Lane M: 1 kb DNA size marker

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.

Acknowledgement

This study was financially supported by the Student Research Committee (SRC), Research Council of Mashhad University of Medical Sciences, Mashhad, Iran (Grant No. 931063).

References

1. Kaufmann SH. Fact and fiction in tuberculosis vaccine research: 10 years later. Lancet Infect Dis. 2011;11(8):633-40.

2. D'Souza S, Denis O, Scorza T, Nzabintwali F, Verschueren H, Huygen K. CD4+ T cells contain Mycobacterium tuberculosis infection in the absence of CD8+ T cells in mice vaccinated with DNA encoding Ag85A. Eur J Immunol. 2000;30(9):2455-9.

3. Akhavan R, Meshkat Z, Jamehdar S. Comparing the Frequency of Mycobacterium Tuberculosis With Direct Microscopy and Culture Methods. Jundishapur J Microbiol. 2012;6(1):95-6.

4. Baghani A, Youssefi M, Safdari H, Teimourpour R, Meshkat Z. Designing and Construction Pcdna3.1 Vector Encoding Cfp10 Gene of Mycobacterium tuberculosis. Jundishapur J Microbiol. 2015;8(10).

5. Organization WH. Global tuberculosis control: WHO report 2010: World Health Organization; 2010.

6. Soleimanpour S, Farsiani H, Mosavat A, Ghazvini K, Eydgahi MR, Sankian M, et al. APC targeting enhances immunogenicity of a novel multistage Fc-fusion tuberculosis vaccine in mice. Appl Microbiol Biotechnol. 2015 Dec; 99 (24):10467-80.

7. Romano M, Aryan E, Korf H, Bruffaerts N, Franken C, Ottenhoff T, et al. Potential of Mycobacterium tuberculosis resuscitation-promoting factors as antigens in novel tuberculosis sub-unit vaccines. Microbes Infect. 2012;14 (1):86-95.

8. Gupta UD, Katoch VM, McMurray DN. Current status of TB vaccines. Vaccine. 2007; 25(19):3742-51.

9. Wieczorek AE, Troudt JL, Knabenbauer P, Taylor J, Pavlicek RL, Karls R, et al. HspX vaccination and role in virulence in the guinea pig model of tuberculosis. Pathog Dis. 2014 Aug; 71(3):315-25.

10. de Sousa EM, da Costa AC, Trentini MM, de Araújo Filho JA, Kipnis A, Junqueira-Kipnis AP. Immunogenicity of a fusion protein containing immunodominant epitopes of Ag85C, MPT51, and HspX from Mycobacterium tuberculosis in mice and active TB infection. PloS one. 2012; 7(10):e47781.

11. Taylor JL, Wieczorek A, Keyser AR, Grover A, Flinkstrom R, Karls RK, et al. HspX-mediated protection against tuberculosis depends on its chaperoning of a mycobacterial molecule. Immunol Cell Biol. 2012;90(10):945-54.

12. Teimourpour R, Sadeghian A, Meshkat Z, Esmaelizad M, Sankian M, Jabbari AR. Construction of a DNA Vaccine Encoding Mtb32C and HBHA Genes of Mycobacterium tuberculosis. Jundishapur J Microbiol. 2015; 8(8):e21556.

13. Baghani A, Youssefi M, Safdari H, Teimourpour R, Meshkat Z. Designing and Construction Pcdna3. 1 Vector Encoding Cfp10 Gene of Mycobacterium tuberculosis. Jundishapur J Microbiol. 2015;8(10).

14. Radošević K, Wieland CW, Rodriguez A, Weverling GJ, Mintardjo R, Gillissen G, et al. Protective immune responses to a recombinant adenovirus type 35 tuberculosis vaccine in two mouse strains: CD4 and CD8 T-cell epitope mapping and role of gamma interferon. Infect Immun. 2007;75(8):4105-15.

15. Yuan W, Dong N, Zhang L, Liu J, Lin S, Xiang Z, et al. Immunogenicity and protective efficacy of a tuberculosis DNA vaccine expressing a fusion protein of Ag85B-Esat6-HspX in mice. Vaccine. 2012;30(14):2490-7.

16. Zhang X, Divangahi M, Ngai P, Santosuosso M, Millar J, Zganiacz A, et al. Intramuscular immunization with a monogenic plasmid DNA tuberculosis vaccine: enhanced immunogenicity by electroporation and co-expression of GM-CSF transgene. Vaccine. 2007;25(7):1342-52.

17. Barker LF, Brennan MJ, Rosenstein PK, Sadoff JC. Tuberculosis vaccine research: the impact of immunology. Curr Opin Immunol. 2009;21(3):331-8.

18. Girard MP, Fruth U, Kieny M-P. A review of vaccine research and development: tuberculosis. Vaccine. 2005;23(50):5725-31.

19. Hu Y, Movahedzadeh F, Stoker NG, Coates AR. Deletion of the Mycobacterium tuberculosis α-crystallin-like hspX gene causes increased bacterial growth in vivo. Infect Immun. 2006; 74(2):861-8.

20. Chauhan P, Jain R, Dey B, Tyagi AK. Adjunctive immunotherapy with α-crystallin based DNA vaccination reduces Tuberculosis chemotherapy period in chronically infected mice. Sci Rep. 2013; 3:1821.
21. Redchuk T, Korotkevich N, Gorbatiuk O, Gilchuk P, Kaberniuk A, Oliynyk O, et al. Expression of Mycobacterium tuberculosis proteins MPT63 and MPT83 as a fusion: purification, refolding and immunological characterization. J Appl Biomed. 2012; 10(4):169-76.
22. Shi C, Chen L, Chen Z, Zhang Y, Zhou Z, Lu J, et al. Enhanced protection against tuberculosis by vaccination with recombinant BCG over-expressing HspX protein. Vaccine. 2010; 28(32):5237-44.
23. Niu H, Hu L, Li Q, Da Z, Wang B, Tang K, et al. Construction and evaluation of a multistage Mycobacterium tuberculosis subunit vaccine candidate Mtb10. 4–HspX. Vaccine. 2011; 29(51):9451-8.
24. Haldar S, Sankhyan N, Sharma N, Bansal A, Jain V, Gupta V, et al. Detection of Mycobacterium tuberculosis GlcB or HspX Antigens or devR DNA Impacts the Rapid Diagnosis of Tuberculous Meningitis in Children. PloS one. 2012; 7(9):e44630.
25. Mir F-A, Kaufmann SH, Eddine AN. A multicistronic DNA vaccine induces significant protection against tuberculosis in mice and offers flexibility in the expressed antigen repertoire. Clin Vaccine Immunol. 2009; 16(10):1467-75.