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Designing and Construction of a Cloning Vector Encoding *mtb32C and mpt51* Fragments of *Mycobacterium tuberculosis* as a DNA Vaccine Candidate

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ABSTRACT

Background & Objective: Tuberculosis (TB) remains a major cause of death around the world. Bacillus Calmette Guérin (BCG) is the only vaccine used in TB prevention that has a protective effect in children, but its effectiveness declines in adults. Design and development of new vaccines is the most effective way against TB.

The aim of this study was to design and construct a DNA vaccine encoding *mtb32C* and *mpt51* fusion genes of *Mycobacterium tuberculosis*.

Methods: First, *mpt51* fragment was amplified by PCR method. The pcDNA3.1+/*mtb32C* plasmid was transformed into *E. coli* JM109 and then extracted. The *mpt51* gene and pcDNA3.1+/*mtb32C* plasmid were both digested with *Eco*RI and *Bam*HI restriction enzymes followed by ligation of *mpt*51 fragment into the digested vector. The recombinant plasmid containing *mtb32C* and *mpt51* was subsequently transformed into competent *E. coli* TOP10 strain. The clones were confirmed by colony-PCR, restriction enzyme digestion and sequencing.

Results: Using agarose gel electrophoresis, a 926 bp fragment corresponded to *mpt51* was observed. Digestion of the vector pcDNa3.1+/*mtb32C* and *mpt51* gene was confirmed by electrophoresis. Then, the pcDNA3.1+/*mtb32C* plasmid was extracted. Sequencing results confirmed the accuracy of the desired plasmid.

Conclusion: In this study, we constructed a cloning vector encoding *mtb32C/mpt51* gene of *M. tuberculosis*. The eukaryotic expression of this vector can be confirmed in future studies. It can be considered as a DNA vaccine in animal models later. Successful cloning provides a basis for the development of new DNA vaccines against TB.

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Introduction

Tuberculosis (TB) is a major and contagious infectious disease with over eight million new cases per year (1, 2). According to the World Health Organization (WHO), one-third of the world's population is estimated to be infected with *Mycobacterium tuberculosis* (*M. tuberculosis*). Of these, 5-10% of the latent population will develop clinical manifestations of TB, of which 2-3 million people die, annually (3, 4). Bacillus Calmette Guérin (BCG) vaccine which has been administered to more than one billion people worldwide, has shown its significant effectiveness against tuberculosis meningitis and miliary tuberculosis in youngsters but not in adults (5). On the other hand, in the 1980s, it became clear that those infected with AIDS are susceptible to *M. tuberculosis* infection. These reasons and the higher risk of disseminated BCG tuberculosis in HIV-infected neonates encouraged scientists to develop new vaccines against tuberculosis. These vaccines should stimulate Th1 cells to increase the capacity of macrophages against

the disease (6).

MTB32A is a serine protease extracted from culture filtrate proteins (CFP) of *M. tuberculosis*. This protein plays a major role in the bacterial survival and pathogenesis. The C-terminal of MTB32A is capable of eliciting TCD8+ and resistance to TB (7).

Mpt51 is a major secreted protein of the *M. tuberculosis*, which is found in the genomes of *M. tuberculosis*, *M. leprae*, *M. avium*, and *M. bovis* BCG. This protein attaches to the fibronectin of the extracellular matrix of the host cells and might have a major role in the virulence of the bacterium (8).

Mpt51 is closely related to the Ag85 complex of *M. tuberculosis* and has a high tendency towards fibronectin. It has mycolyl transferase activity that is necessary to make trehalosedimycolate (cord factor), and a structure necessary to maintain the integrity of the cell wall (9). The evidences suggest that mpt51 plays an important role in adhering to host tissue through binding to fibronectin (10). The aim of this study was to design and construct a DNA vaccine encoding *mtb32C* and *mpt51* fusion genes of *M. tuberculosis*.

Materials and Methods

Bacterial strains and DNA extraction

Standard strain of *M. tuberculosis* H37Rv (ATCC 27294) was purchased from Tuberculosis Reference Laboratory of Shariati Hospital (North-East of Mashhad) and cultured on Löwen stein–Jensen medium at 37ºC for a period of four weeks. Then, DNA was extracted by boiling procedure; in brief, 4-5 colonies of *M .tuberculosis* H37Rv were suspended and homogenized in 500 µl sterile PBS buffer. Suspended bacteria were boiled for 15 min. The supernatant was used as DNA template in the PCR reaction (11).

Primer designing and PCR method

DNA encoding *mpt51* sequence is located in RD1 region and called *mpt51*, *mpb51*, *fbpC1*or Rv3803c. To develop specific primers for the amplification of *mpt51*, the DNA fragment encoding *mpt51* gene consisting of 926 nucleotides was retrieved from NCBI GenBank. Based on this sequence, the forward and reverse primers; (5’ -AGATAAGGATCCACCATGGGAAAGGGTCGGTCGGCGCT-3’) (5’-TCGGCAGAATTCTTAGCGGATCGCACCGACGATATCGC-3’) were designed using Gene Runner software. The PCR mixture contained 10X PCR buffer, 0.1mM dNTP, 1.5 mM $MgCl_2$, 1 µl DNA (100ng/µl), 10 pmol of each primer, 1.5 unit of *Taq* DNA polymerase (Fermentas, UK).The adequate amount of double distilled water (DDW) was added to the reaction. The negative control contained all the PCR reagents without the template DNA. Amplification was done on an Eppendorf thermocycler (UK) using the following cycling conditions: 95°C for 30 sec, 35 cycles at 57°C for 45 sec, 72°C for 30 sec followed by a final extension at 72°C for 3 min. The PCR product electrophoresis was performed on low melting 1% agarose gel (Fermentas, UK). The desired band was purified by DNA extraction kit according to the manufacturer’s protocol (Bioneer, Korea).

Construction of recombinant plasmid *pcDNA3.1+/mtb32C/mpt51:* Digestion, ligation and transformation

PcDNA3.1+ vector containing *mtb32C* (pcDNA3.1+/*mtb32C*) was previously constructed in our laboratory (12). The vector was transformed into *E. coli* JM109 and then purified using plasmid extraction kit (Bioneer, Korea).

Both purified PCR product and pcDNA3.1+/*mtb32C* vector were digested by *Eco*RI and *Bam*HI restriction enzymes (Fermentas, UK). The reaction mixture contained 20 µl of *mpt51* DNA (25 ng/µl), 5 µl H Buffer, 2 µl *Eco*RI (10 u/µl), 2 µl *Bam*HI (10 u/µl) and 31 µl nuclease-free DDW. The vector concentration for enzyme digestion was 100 ng/µl. The digested products were run on the gel and purified by gel extraction kit (Bioneer, Korea). In order to insert the *mpt51* gene into pcDNA3.1+/*mtb32C* vector, ligation was performed with *T4 DNA ligase* (Fermentas, UK). The ligation reaction included: 6 µl (100 ng/µl) of pcDNA3.1+/*mtb32C*, 12 µl (100 ng/µl) of *mpt51*, 2.5 µl of *T4 DNA ligase* buffer, 2 µl of 5u/µl *T4 DNA ligase*, 2 µl of polyethylene glycol (PEG 4000) and 0.5 µl of DDW. The competent *E. coli* TOP10 strain was prepared using cold solution of $CaCl_2$/$MgCl_2$ (0.1

M) as described previously (12, 13). Transformation was performed using heat shock method and the transformed bacteria were cultured on LB agar containing 100 µg/ml ampicillin (14).

Confirming the desired construct

The colonies grown on the LB agar plates containing ampicillin were used as DNA template in PCR reaction using *mpt51* gene primers with the same conditions that were optimized for *mpt51* gene. The accuracy of designed pcDNA3.1+/*mtb32C*/*mpt51* recombinant vector was confirmed by sequencing.

Results

The amplification of *mpt51* gene was performed by the PCR method using specific primers and a fragment with 926 bp in length was observed on 1% w/v agarose gel electrophoresis (Figure1).

The PCR product band related to *mpt51* gene was purified (Figure2). Recombinant vector pcDNA3.1+/*mtb32C* and *mpt51* gene were digested with *Eco*RI and *Bam*HI restriction enzymes, then ligation was performed using *T4 DNA ligase* enzyme. The ligation product was transformed into competent *E. coli* TOP10 strain (7, 15). The colony PCR was performed to select recombinant vectors containing *mpt51* gene (Figure 3). The sequencing results showed 100% sequence homology without any gap and mutation in our final recombinant plasmid compared to recorded sequences of H37Rv *mtb32C* and *mpt51* fragments.

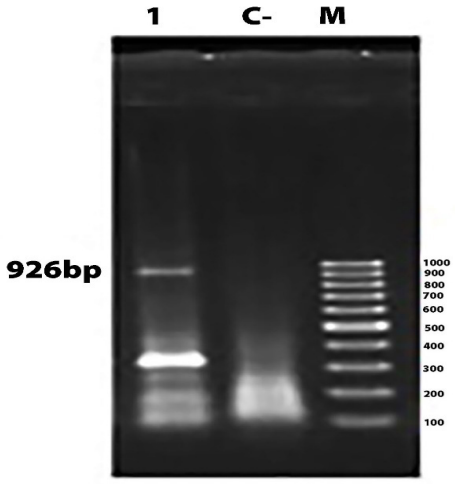

Figure 1. Agarose gel electrophoresis of PCR product showing the amplification of *mpt51* gene. Lane 1: band of 926 bp corresponds to *mpt51*gene; Lane C-: negative control; Lane M: 100 bp DNA size marker (Fermentas, UK).

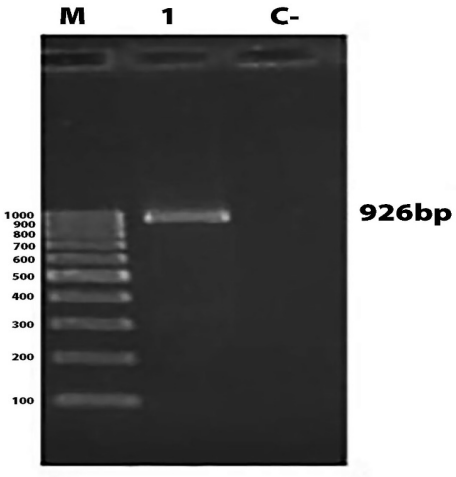

Figure 2. Agarose gel electrophoresis of purified PCR product of 926 bp *mpt51* using DNA extraction kit from gel, Lane M: 100bp DNA size marker (Fermentas, UK); lane 1: purified *mpt51*gene; Lane C-: negative control.

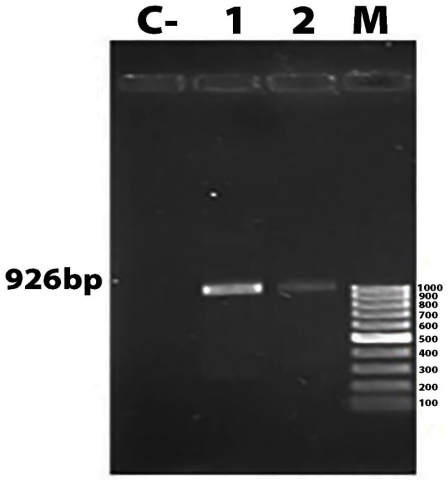

Figure 3. Agarose gel electrophoresis of colony-PCR showing recombinant vector pcDNA3.1+/*mtb32C/ mpt51* using *mpt5*1-specific primers. Lane C-: negative control; Lanes 1 and 2: positive colonies; Lane M: 100 bp DNA size marker (Fermentas, UK).

Discussion

The previous attempts using alternative antigens of mycobacteria other than BCG have not been acceptable. The availability of cloned genes and suitable vectors has paved a new way to synthesize mycobacterial protein antigens individually by transfection to mammalian cells (16).

Studies have shown that, DNA vaccination of heavily infected mice with TB can alter the inefficient immune response to an effective one that destroys bacteria. Immunotherapy with DNA vaccine in combination with conventional antibacterial therapy might result in more efficient treatment of infection in humans. The main effects of this vaccination were stimulation of CD4+ T cells and production of IFN-γ together. As a result, those antigens which are more capable of stimulating T cell responses against *M. tuberculosis* would be more immunogenic, hence, a better candidate for vaccine development. The earlier studies have shown that recombinant fusion protein mtb32/mtb39 vaccine from *M. tuberculosis* provide better immune responses compared to each of the proteins alone. Thus, as a candidate vaccine, the cloning of various antigens with *mtb32* may improve the host immune responses compared to any single antigen (7).

For this purpose, designing and construction of two other fusion DNA vaccines: one encoding *tb10.4* and *mtb32C-hbha* and another encoding ag85A and *tb10.4* fusion of *M. tuberculosis* were conducted in Antimicrobial Resistance Research Center at the University of Mashhad (7, 17).

Conclusion

In this study, *mtb32C/mpt51* recombinant vector was constructed successfully. It should be noted that the termination codon of *mtb32C* has been removed. The gene encoding *mpt51* was added in frame to *mtb32C* sequence. More broadly, research is needed to determine immune responses to this vaccine in animal models.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

References

1. Fang CM, Zainuddin ZF, Musa M, Thong KL. Cloning, expression, and purification of recombinant protein from a single synthetic multivalent construct of Mycobacterium tuberculosis. Protein Expr Purif. 2006;47(2):341-7. https://doi.org/10.1016/j.pep.2005.12.007 PMID:16510294
2. Akhavan R, Meshkat Z, Jamehdar S. Comparing the Frequency of Mycobacterium Tuberculosis With Direct Microscopy and Culture Methods. Jundishapur Journal of Microbiology. 2012;6(1):95-6. https://doi.org/10.5812/jjm.4596
3. Moreno-Mendieta SA, Rocha-Zavaleta L, Rodriguez-Sanoja R. Adjuvants in tuberculosis vaccine development. FEMS Immunol Med Microbiol. 2010;58(1):75-84. https://doi.org/10.1111/j.1574-695X.2009.00629.x PMID:20002177
4. Asnaashari AMH, Sadrizadeh A, Ahmadi H, Meshkat M, Gholoobi A, Talab FR, et al. The study of Mycobacterium tuberculosis in Iranian patients with lung cancer. Jundishapur J Microbiol. 2013;6(3):237-41. http://dx.doi.org/10.5812/jjm.4993
5. Taniguchi K, Takii T, Yamamoto S, Maeyama J-i, Iho S, Maruyama M, et al. Reactivation of immune responses against Mycobacterium tuberculosis by boosting with

the CpG oligomer in aged mice primarily vaccinated with Mycobacterium bovis BCG. Immun Ageing. 2013;10:25. https://dx.doi.org/10.1186%2F1742-4933-10-25 PMID:23799936

6. Kaufmann SH, Hussey G, Lambert P-H. New vaccines for tuberculosis. Lancet. 2010; 375(9731): 2110-9. https://doi.org/10.1016/S0140-6736(10)60393-5 PMID:20488515
7. 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. https://doi.org/10.5812/jjm.21556 PMID: 26464766
8. Silva BD, da Silva EB, do Nascimento IP, Dos Reis MC, Kipnis A, Junqueira-Kipnis AP. MPT-51/CpG DNA vaccine protects mice against Mycobacterium tuberculosis. Vaccine. 2009;27(33):4402-7. https://doi.org/10.1016/j.vaccine.2009.05.049 PMID: 19500525
9. Sundar S, Annaraj D, Selvan A, Biswas PG, Vijayakumaran R, Anishetty S. Functional insights from a comparative study on the dynamics of Antigen85 proteins and MPT51 from Mycobacterium tuberculosis. J Mol Model. 2015;21(12):310. https://doi.org/10.1007/s00894-015-2853-4 PMID:26564147
10. Wilson RA, Maughan WN, Kremer L, Besra GS, Fütterer K. the structure of Mycobacterium tuberculosis MPT51 (FbpC1) defines a new family of non-catalytic α/β hydrolases. J Mol Biol. 2004;335(2):519-30. https://doi.org/10.1016/j.jmb.2003.11.001 PMID: 14672660
11. Gholoobi A, Masoudi-Kazemabad A, Meshkat M, Meshkat Z. Comparison of culture and PCR Methods for diagnosis of mycobacterium tuberculosis in different clinical specimens. Jundishapur J Microbiol. 2014;7(2): e8939. https://dx.doi.org/10.5812%2Fjjm.8939 PMID: 25147673
12. Nabavinia MS, Nasab MN, Meshkat Z, Derakhshan M, Khaje-Karamadini M. Construction and evaluation of an expression vector containing Mtb32C (Rv0125) of Mycobacterium tuberculosis. Avicenna J Medl Biotechnol. 2011;3(4): 207–210. PMID: 23407610
13. Lew JM, Kapopoulou A, Jones LM, Cole ST. TubercuList–10 years after. Tuberculosis. 2011;91(1):1-7. https://doi.org/10.1016/j.tube.2010.09.008 PMID: 20980199
14. 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): e23560. PMID: 26587210
15. Rashidian S, Teimourpour R, Meshkat Z. Designing and construction of a DNA vaccine encoding tb10. 4 gene of Mycobacterium tuberculosis. Iran J Pathol. 2016;11(2): 112–119. PMID: 27499771
16. Nabavinia MS, Nasab MN, Meshkat Z, Derakhshan M, Khaje-Karamadini M. Construction of an expression vector containing Mtb72F of mycobacterium tuberculosis. Cell J. 2012;14(1):61-66. PMID: 23626939
17. Meshkat Z, Teimourpour A, Rashidian S, Arzanlou M, Teimourpour R. Immunogenicity of a DNA Vaccine Encoding Ag85a-Tb10. 4 Antigens from Mycobacterium Tuberculosis. Iran J Immunol. 2016;13(4):289-295. PMID: 27999240

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