

Evaluation of the eukaryotic expression of *mtb32C-hbha* fusion gene of *Mycobacterium tuberculosis* in Hepatocarcinoma cell line

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Materials and Methods: In present study Huh7.5 cells was transfected with Mtb32C-HBHA –pCDNA3.1+ recombinant vector by using the calcium phosphate method and expression of chimeric protein was assessed by RT-PCR and Western blot methods.

Background and Objective: HBHA and Mtb32C have been isolated from culture supernatants of *Mycobacterium tuberculosis* (*M. tuberculosis*) and *Mycobacterium bovis* (*M. bovis*) and their immunogenicity in previous studies have been confirmed. In this study, capability of constructed vector containing two mycobacterial immunodominant antigens (Mtb32C-HBHA), in producing new chimeric protein under the *in-vitro* condition was examined.

Results: Recombinant vector introduce to Huh7.5 cell and generation of recombinant Mtb32C-HBHA protein was evaluated by RT-PCR and Western blot methods. Results of RT-PCR and Western blot showed expression of 35.5 KD recombinant protein (Mtb32C-HBHA) in this cell line(Fig1,2,3).

Conclusion:

This study indicated, the constructed vector can produce two highly immunogenic antigens that fusion of them to gather makes chimeric antigen with new traits. Other attempts are needed to evaluate specific properties of this new antigen such as molecular conformation modeling and immunologic characteristics in future studies.

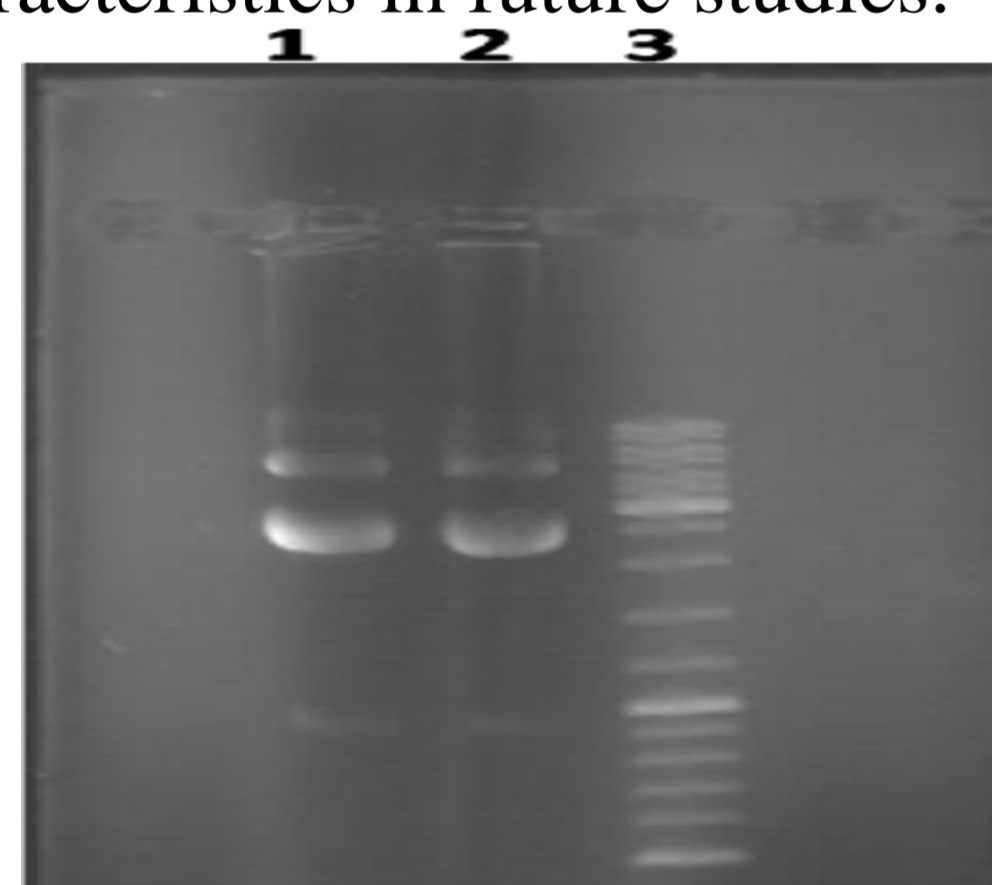


Fig.1: Gel electrophoresis of the recombinant vector encoding *mtb32C-hbha* fusion gene, after purification. Lanes 1 and 2: purified recombinant vector containing *mtb32C-hbha* genes; lane3: 100bp DNA size marker (Fermentas, Germany)

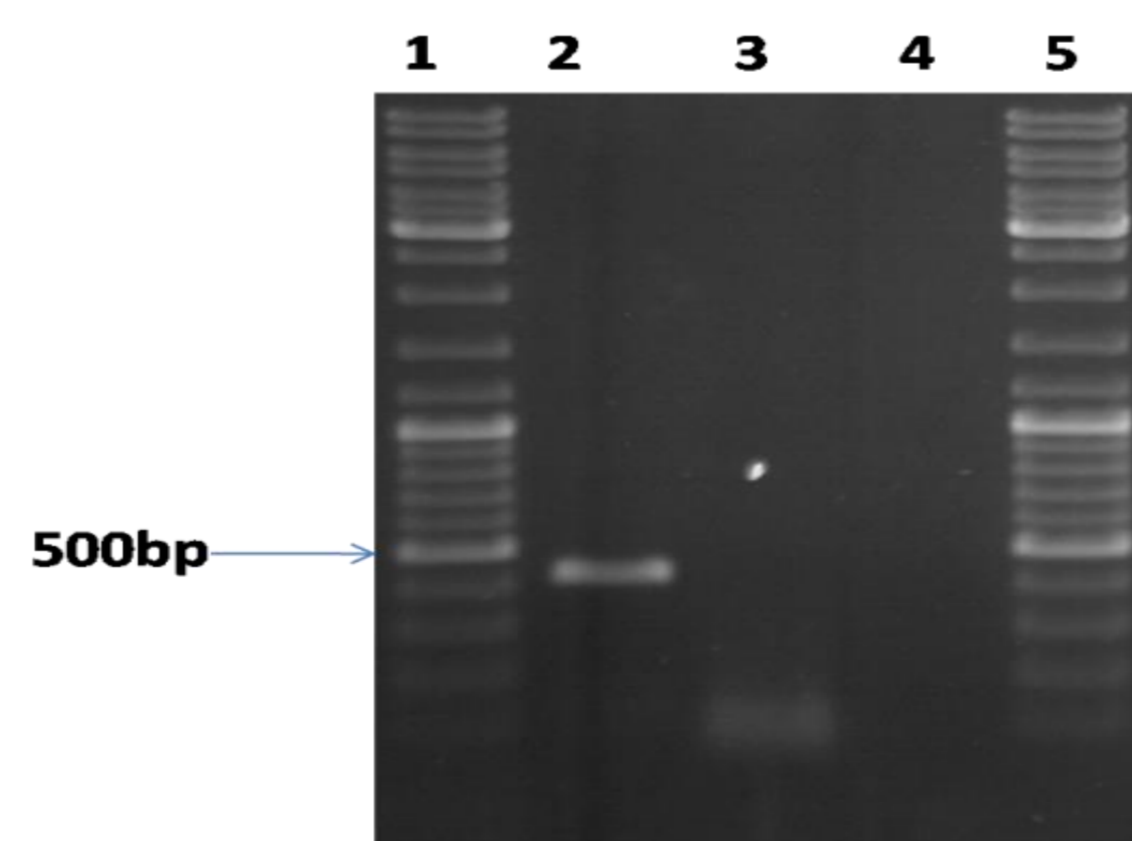


Figure 2: Identification of Mtb32C mRNA in transfected and non-transfected Huh-7.5 cells by RT-PCR method. A 400bp fragment was amplified by RT-PCR method in transfected cells (lane 2); no amplification was resulted by RT-PCR on extracted RNA treated with DNase I (lane 3) and on synthesized cDNA of non transfected cells (lane 4). Lanes 1 and 5: 100bp DNA size marker (Fermentas, Germany)

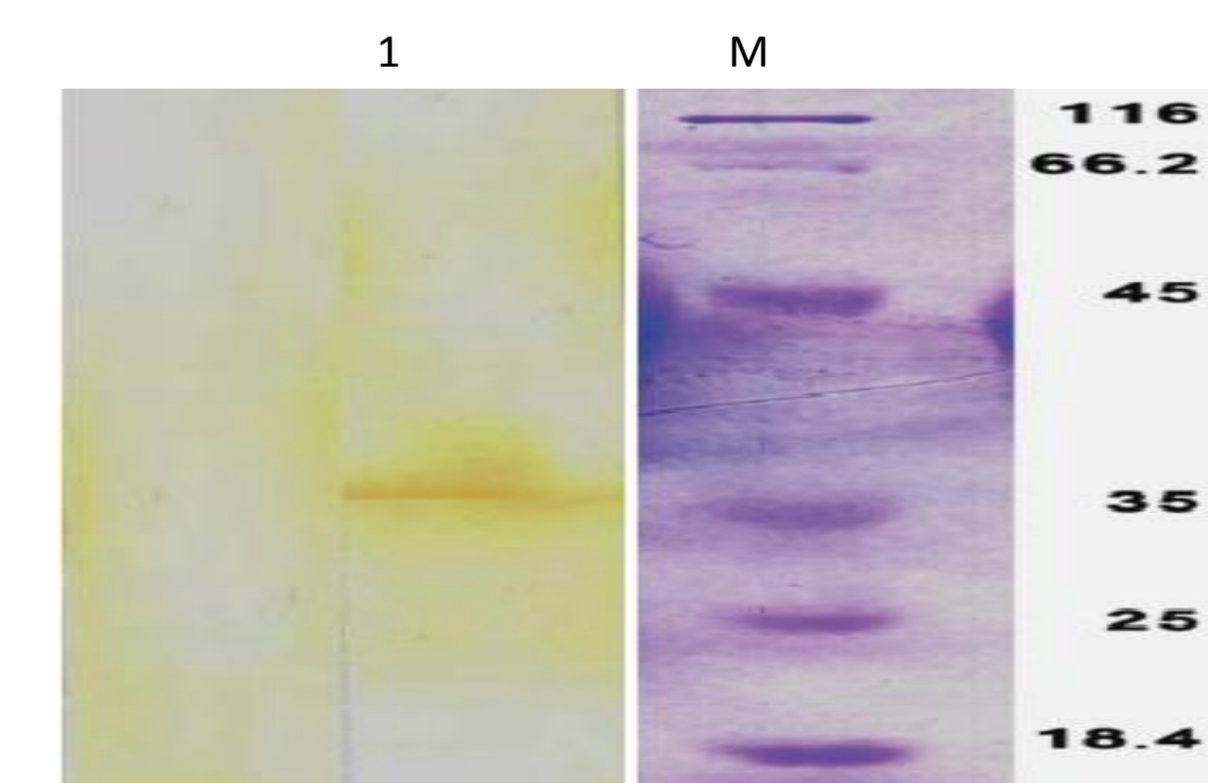


Fig 3: Recognition of an approximately 35.5 kDa band corresponding to Mtb32C-HBHA fusion protein in transfected cells (Lane 1) and non-transfected cells (Lane 2) by Western blot method

References

1. Akhavan R, Meshkat Z, Khajekaramadini M, Meshkat M. Eight-year study of *Mycobacterium tuberculosis* in mashhad, northeast of iran. *Iranian Journal of Pathology*. 2013;8(2):73-80.
2. Mandell D, and Bennett. *Principles and Practice of Infectious Diseases*. 8 ed: Saunders; 2014.
3. Organization WH. *Global tuberculosis report 2014*. Available from: www.who.int/tb.
4. BW M. Topley and Wilson's *Microbiology and Microbial Infections*. 10 ed. London: wiley; 2005.
5. Pym AS, Brodin P, Brosch R, Huerre M, Cole ST. Loss of RD1 contributed to the attenuation of the live tuberculosis vaccines *Mycobacterium bovis* BCG and *Mycobacterium microti*. *Mol Microbiol*. 2002;46(3):709-17.