Original Article

Cloning and Expression of Helicobacter pylori HpaA Gene

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Abstract

Received: 14/Sep/2008, Accepted: 22/Nov/2008

Objective: Helicobacter pylori is associated with chronic gastritis, peptic ulcers, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Antibiotic therapies do not protect from potential re-infection and have a risk for development of drug resistance. Therefore, prophylactic vaccine mediated protection against H. pylori is an attractive clinical interest. H. pylori adhesin A (HpaA) is a conserved surface lipoprotein and plays important roles in the pathogenesis of infection. In this study the recombinant protein (rHpaA) was over-expressed in E.coli.

Materials and Methods: The hpaA gene was amplified by PCR. Prokaryote expression vector pET28a-hpaA was constructed, and used to transform E.coli BL21DE3. The expression of recombinant protein induced by IPTG was examined by SDS-PAGE. Western blot were used to determine immunoreactivity of rHpaA by a rabbit polyclonal antibodies against whole cell of H. pylori.

Results: The *hpaA* gene nucleotide sequence in the recombinant plasmid vector of pET-$_{28a}$-*hpaA* was consistent with that of H.pylori *hpaA* as published in the GenBank. SDS-PAGE demonstrated that the constructed prokaryotic expression efficiently produced rHpaA at the 1.5 mmol/L of IPTG. HpaA fusion protein was able to react with the rabbit polyclonal antibody against whole cells of H. pylori.

Conclusion: A prokaryotic expression system pET-$_{28a}$-*hpaA*-BL21 with high efficiency of H. pylori *hpaA* gene was successfully established and the HpaA fusion protein showed satisfactory immunoreactivity. These results indicate that production of a specific recombinant protein is an alternative and potentially more expeditious strategy for development of H. pylori vaccine.

Keywords: Helicobacter pylori, *hpaA*, Recombinant Protein, pET$_{28a}$

Yakhteh Medical Journal, Vol 11, No 3, Autumn 2009, Pages: 273-276

Introduction

Helicobacter pylori (H. pylori) is a gram-negative, extracellular, microaerophilic spiral bacterium that colonizes as much as 50% of the human gastric mucosa worldwide. H. pylori infection commonly results in asymptomatic chronic gastritis, but some infected individuals develop peptic ulcers, gastric adenocarcinoma or mucosa-associated lymphoid tissue (MALT) lymphoma (1-3). Treatment often consists of various medicines (antibiotics and proton pump inhibitors), but the cost of combination therapy and the emergency of antibiotic resistance have led to great interest for vaccine development (1, 4). Additionally, natural H. pylori infection does not generate a protective immunity and re-infection occasionally can occur (5, 6).

In regards to the virulence factors; motility plays an important role in H. pylori pathogenesis. A bundle of three to six flagella that extends from one pole of the bacterium confers a high degree of motility (7, 8). The flagella have a membranous sheath that envelops each filament. H. pylori adhesion protein A (HpaA), a sheath protein with approximately 29KD$_a$ located in the outer membrane of bacterium, plays an important role in adhesion of H. pylori (9, 10). Thus, in this study, a plasmid recombinant containing *hpaA* gene was constructed and the immunogenicity of recombinant HpaA protein was examined.

Materials and Methods

Bacterial strains and plasmid

The PCR experiments were performed with genom-

ic DNA prepared from H. Pylori, ATTCC 26695. E.coli DH5α and E. coli BL21DE3 were used for cloning and expression experiments. Plasmid pET-28a was used as a cloning and expression vector.

Amplification of hpaA gene

H. pylori was cultured on H. pylori-selective agar plates with 10% defibrillated sheep blood and antibiotics at 37°C under microaerobic conditions (10% CO_2, 5% O_2 and 85% N_2). Genomic DNA was extracted by a routine phenol-chloroform method (11). The DNA fragment coding for *hpaA* gene was amplified by using oligonucletides Hpa1 (5'-GTAGGATCCATGAAAGCAAATAATCATTTTAAAG-3') as a forward primer with an endonuclease site of BamHI and Hpa2 (5'-GTAAAGC TTTTATCGGG TTTCTTTTGCCT-3') as reverse primer with an endonuclease site of *HindIII*. Amplification was made in a total volume of 50 µl of reaction mixture containing 10 µl of 10× PCR buffer, 2.5 mM $MgCl_2$, 200 µM dNTP, 1.25 units of Taq polymerase, 20 pmol of each primer and 1 µl of sample DNA under conditions: 94°C for 5 minutes, then 30 cycles at 94°C for 1 minute, 58°C for 1 minute and 72°C for 1 minute, followed by 10 minutes at 72°C. The PCR products were analyzed on 1% agarose gels stained with ethidium bromide. The expected size of target amplification fragment was 801bp.

Cloning and sequencing

Fragments of *BamHI* and *HindIII*-digested *hpaA* were inserted into the *BamHI/HindIII* site of expression vector pET-28a, through enzyme digestion and ligation reactions. Then the recombinant plasmid pET-28a-*hpaA* was confirmed by PCR and restriction enzyme digestion. Recombinant pET-28a-*hpaA* was amplified in competent E.coli DH5α, and then extracted by Sambrook's method (11). The resulting plasmid pET-28a-*hpaA* was transformed into competent final host E. coli BL21 DE3 and kanamycin resistance was used for selection (11). A large scale recombinant plasmid was prepared and identified by restriction enzymes. DNA sequence was performed with a DNA automatic sequencer.

Expression and identification of the fusion protein

The *hpaA* expression system pET-28a -*hpaA*-BL21DE3 was cultured in LB medium at 20°C and induced by isopropylthio-β-D-galactoside (IPTG) at different concentrations of 1.5, 1 and 0.5mmol/L. The precipitate was isolated by centrifugation and the cell pellet was broken by 100 µl loading buffer. The molecular weight of HpaA fusion protein was identified by separation of whole-cell lysates by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Immunoblot analysis

Immunoreactivity of HpaA fusion protein was determined by Western blot. For Western blotting, proteins were transferred to nitrocellulose sheets and incubated with the self-prepared rabbit antiserum against whole cell H. pylori and HRP-labeling sheep anti-rabbit IgG as the first and second antibodies, respectively.

Results

Construction of recombinant pET-28a-hpaA

The PCR product amplified from genomic DNA of H. pylori strain 26695 is shown Fig 1. The 801-bp expected fragment amplified by PCR contained a gene *hpaA*. The 801-bp PCR product was digested with *BamHI* and *HindIII* restriction enzyme and ligated into the corresponding sites of pET-28a. The recombinant plasmids pET-28-a-*hpaA* were digested by *BamHI* and *HindIII* and analyzed on agarose gel electrophoresis (Fig 2).

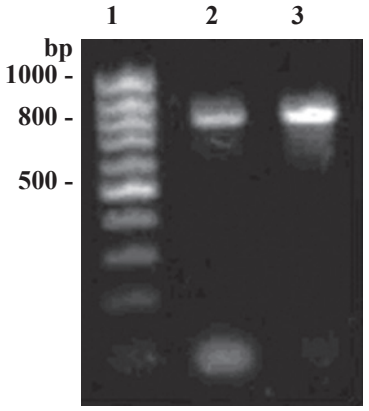

Fig 1: The target fragment of hpaA gene amplified from H. pylori strain 26695. Lane1: 100bp DNA marker, Lanes 2 & 3: The target amplification of hpaA gene.

Nucleotide sequence analysis

The *hpaA* gene nucleotide sequence in the recombinant plasmid vector of pET-28a-*hpaA* was consistent with that of H. pylori *hpaA* as published in the GenBank (12).

Expression of recombinant fusion protein

The recombinant pET-28a-*hpaA* was transformed into BL21 E.coli strains and the fusion protein was expressed. The 1.5 mmol/L of IPTG was able to efficiently induce expression of HpaA fusion protein with a predicted molecular mass of 30KD (Fig 3).

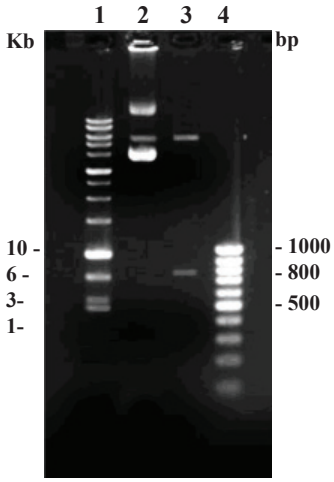

Fig 2: Agarose gel electrophoresis analysis of recombinant pET-28a-hpaA. Lane1:1Kb DNA marker, Lane 2: pET-28a without insertion, Lane 3: Double digest of recombinant pET-28a-hpaA with BamHI and HindIII, Lane 4: 100bp DNA marker.

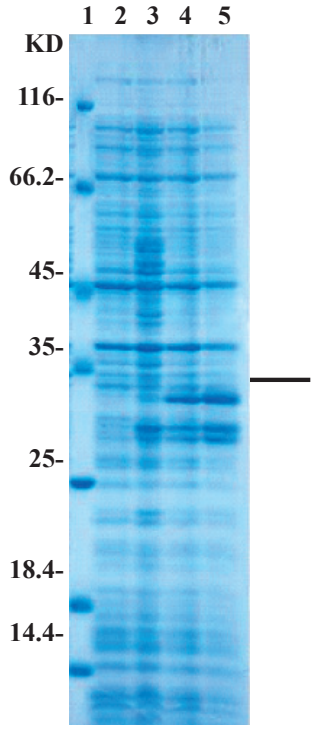

Fig 3: Expression of HpaA protein in pET-28a-hpaA-BL21. Lane1: Protein marker, Lane 2: Bacterial cell without plasmid, Lane 3: Non-induced recombinant bacterial cells, Lane 4: Induced bacterial cells 1 hour, Lane 5: Induced bacterial cells 4 hours.

Antigenicity study of HpaA fusion protein

Sera were obtained from a rabbit immunized with H. pylori. The recombinant fusion protein was recognized by the rabbit antiserum against the whole cell of H. pylori. Analysis of recombinant HpaA fusion protein by Western blotting is shown in Fig 4.

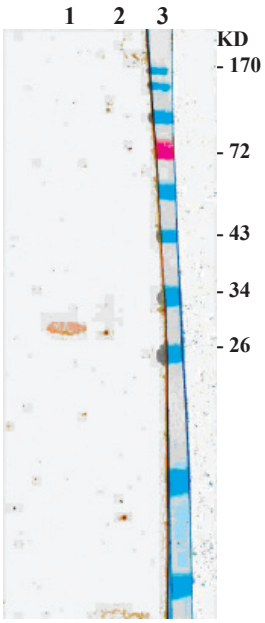

Fig 4: Western blotting of expressed pET-28a-hpaA products. Lane1: Induced recombinant bacterial cells, Lane 2: Non-induced recombinant bacterial cells, Lane 3: Protein marker.

Discussion

The outer membrane proteins in gram negative bacteria have particular significance as a potential target for protective immunity. H. pylori adhesion protein A (HpaA) is one of the major structural outer membrane proteins of H. pylori and plays an important role in adhesion of the bacterium (5, 13). HpaA protein is located both on the bacterial surface and on the flagellar sheath (14). HpaA is a highly conserved protein among H. pylori isolates and found to be produced by all H. pylori isolates tested (13, 15). Previous studies have shown that HpaA is able to induce maturation of human dendritic cells and is essential for colonization of H. pylori in mice (9,10). Furthermore, genomic studies have shown no significant sequence homology of HpaA with other known proteins and antibody against HpaA could be found in approximately 86% of H. pylori infected patients. This proportion is similar to that of urease B and higher than that of vacuolating cytotoxin and heat shock protein (68%) (12, 15). It has been shown that oral immunization of H. pylori infected mice with HpaA induced a significant reduction in bacterial load in the stomach, and produced specific mucosal CD4+ T cell responses with a Th1 profile as well as mucosal IgA responses locally in the stomach (16). Therefore, because of its conserved sequence, strong antigenicity, universal distribution in different H pylori isolates and exposure on the surface of the bacterium, HpaA is one of important antigen

candidates for H. pylori vaccine.

Recombinant HpaA protein expressed by the prokaryotic expression systems has been constructed in some previous studies. They have used different methods and vectors for cloning and expression of this gene. Xu and coworkers constructed a live recombinant attenuated Salmonella typhimurium DNA vaccine strain expressing HpaA protein (17). Yan et al. cloned the PCR product of *hpaA* gene from H. pylori in pET-32a a prokaryotic expression vector (18). The pET-32a vector also was used by Mao and colleagues (19). In our study, the *hpaA* gene from H. pylori 26695 was amplified by PCR. The PCR product was cloned in pET-28a and then transformed into E.coli BL 21. The nucleotide sequence of *hpaA* gene cloned in this study was consistent with that of H.pylori *hpaA* as published in the GenBank (12). We used pET-28a because it did not fuse any additional peptides to the target protein, whereas pET-32a has been designed for cloning and expression of peptide sequences fused with the 109aa Trx Tag™ thioredoxin protein (20). In this study, SDS-PAGE demonstrated that the constructed prokaryotic expression pET-28a-*hpaA*-BL21 efficiently produced rHpaA at the 1.5 mmol/L of IPTG. Cloning sites in the pET-28a also contained cleavable His Tag sequences for detection and purification. However, in our study, the rabbit antibody against whole cell H. pylori recognized and combined with HpaA recombinant protein as confirmed by Western blot, which indicated a high immunoreactivity of the fusion protein. The presence of His Tag sequences in the target protein also provide the possibility for purification through Ni-NTA affinity chromatography.

Conclusion

HpaA is one of the important and ideal antigens that can be potentially used for development of an H. pylori vaccine. An HpaA expression system pET-28a-*hpaA*-BL21 with a high efficiency has been successfully constructed in our study. There is no conflict of interest in this article.

References

1. Kusters JG, van Vliet AH, Kuipers EG. Pathogenesis of Helicobacter pylori infection. Clin Microbiol Rev. 2006; 19: 449-490.
2. Moss SF, Sood S. Helicobacter pylori. Curr Opin Infect Dis. 2003; 16: 445-451.
3. Al-Akwaa AM, Siddiqui N, Al-Mofleh IA. Primary gastric lymphoma. World J Gastroenterol. 2004; 10: 5-11.
4. Boyanova L, Mentis A, Gubina M, Rozynek E, Gosciniak G, Kalenic S, et al. The status of antimicrobial resistance of Helicobacter pylori in Eastern Europe. Clin Microbiol Infect. 2002; 8: 388-396.
5. O'Toole PW, Janzon L, Doig P, Huang J,Kostrzynska M, Trust TJ. The putative neuraminyllactose-binding hemagglutinin HpaA of Helicobacter pylori CCUG 17874 is a lipoprotein. J Bacteriol. 1995; 177: 6049-6057.
6. Raghavan S, Svennerholm AM, Holmgren J. Effects of oral vaccination and immunomodulation by cholera toxin on experimental Helicobacter pylori infection, reinfection, and gastritis. Infect Immun. 2002; 70: 4621-4627.
7. Eaton KA, Brooks CL, Morgan DR and Krakowka S. Motility as a factor in the colonization of gnotobiotic piglets by Helicobacter pylori. J Med Microbiol. 1992; 37: 123-127.
8. Luke CJ, Penn CW. Identification of a 29 kDa flagellar sheath protein in Helicobacter pylori using a murine monoclonal antibody. Microbiology. 1995; 141(Pt 3): 597-604.
9. Voland P, Hafsi N, Zeitner M, Laforsch S, Wagner H, Prinz C. Antigenic properties of HpaA and Omp18, two outer membrane proteins of Helicobacter pylori. Infect Immun. 2003; 71: 3837-3843.
10. Carlsohn E, Nystrom J, Bolin I, Nilsson CL, Svennerholm AM. HpaA is essential for Helicobacter pylori colonization in mice. Infect Immun. 2006; 74: 920-926.
11. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning, A Laboratory Manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press. 1989; 1.21-1.52, 2.60-2.80, 7.3-7.35, 9.14-9.22.
12. Alm RA, Ling LS, Moir DT, King BL, Brown ED, Nature Doig PC, et al. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen Helicobacter pylori Nature. 1999; 397: 176-180.
13. Bölin I, Lönroth H, Svennerholm AM. Identification of Helicobacter pylori by immunological dot blot method based on reaction of a species specific monoclonal antibody with a surface-exposed protein. J Clin Microbiol. 1995; 33: 381-384.
14. Lundstrom AM, Blom K, Sundaeus V, Bolin I. HpaA shows variable surface localization but the gene expression is similar in different Helicobacter pylori strains. Microb Pathog. 2001; 31: 143-253.
15. Opazo P, Muller I, Rollan A, Valenzuela P, Yudelevich A, Garciade La Guarda R, et al. Serological response to Helicobacter pylori recombinant antigen in Chilean infected patients with duodenal ulcer, non-ulcer dyspepsia and gastric cancer. APMIS. 1999; 107: 1069-1078.
16. Nystrom J, Svennerholm A. Oral immunization with HpaA affords therapeutic protective immunity against H. pylori that is reflected by specific mucosal immune responses.Vaccine. 2007; 25; 2591-2598.
17. Xu C, Li Z, Du Y, Tu Z, Gong Y, Jin J, et al. Construction of a recombinant attenuated Salmonella typhimurium DNA vaccine carrying Helicobacter pylori hpaA. World J Gastroenterol. 2005; 11(1): 114-117.
18. Yan J, Mao Y, Shao Z. Frequencies of the expression of main protein antigens from Helicobacter pylori isolates and production of specific serum antibodies in infected patients. World J Gastroenterol. 2005; 11(3): 421-425.
19. Mao Y, Yan J, Li L, Li S. Construction of hpaA gene from a clinical isolate of Helicobacter pylori and identification of fusion protein. World J Gastroenterol. 2003; 9(7): 1529-1536.
20. LaVallie ER, DiBlasio EA, Kovacic S, Grant KL, Schendel PF, McCoy JM. A thioredoxin gene fusion expression system that circumvents inclusion body formation in the E. coli cytoplasm. Biotechnology. 1993; 11: 187-193.