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Development of Experimental Platforms to Assess *Helicobacter pylori* HopQ Interaction with Host CEACAM Molecules

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ABSTRACT

Background: *Helicobacter pylori* is an extracellular bacterium responsible for various gastrointestinal diseases, such as peptic ulcers and gastric cancer. It uses multiple mechanisms to colonize the harsh, acidic environment of the stomach and establish its pathogenic processes, mostly through CagA translocation. While cell surface integrin molecules were previously believed to be the main mediators anchoring *H. pylori* and facilitating this process, recent studies highlight the critical role of the interaction between the bacterial adhesin HopQ and host CEACAMs in CagA translocation and subsequent pathogenic signaling.

Methods: Recombinant proteins, including HopQ, HopQ-GFP, HopQ-HRP, and C1ND, were produced via gene cloning, expression, and purification techniques. Ligand-receptor interactions were evaluated using FACS analysis along with antigen- and cell-based ELISA assays.

Results: In this study, we have developed antigen and cell-based platforms using recombinant fusion proteins (HopQ-GFP and HopQ-HRP) that effectively interact with recombinant C1ND, as well as various CEACAM molecules expressed on gastric cell lines (MKN45 and AGS).

Conclusion: These assay platforms enable detailed investigation of the HopQ-CEACAM interaction and supports high-throughput screening of inhibitors, facilitating the identification of potential drugs or vaccine candidates targeting *H. pylori* infection. ***DOI: 10.61882/ibj.5043***

Keywords: Adhesin, Enzyme-linked immunosorbent assay, Ligands, Receptor

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INTRODUCTION

Recent statistics indicate that *H. pylori* infects approximately half of the world's population, with a higher prevalence in developing countries[1]. It is the main cause of gastric-related diseases, particularly peptic ulcers and gastric cancer, which is the fifth most common cancer worldwide[2]. This Gram-negative bacterium utilizes various mechanisms for its pathogenicity. The first step in this process is colonization, which involves binding to the host epithelial cell receptors via the OMPs[3-6]. To date,

List of Abbreviations:
AMP: antimicrobial peptide; **C1ND:** N-terminal domain of human CEACAM1; **CEACAM:** carcinoembryonic antigen-related cell adhesion molecule; **DAB:** 3,3'-diaminobenzidine; **ECL:** enhanced chemiluminescence; **GFP:** green fluorescent protein; ***H. pylori*:** *Helicobacter pylori*; **HopQ:** *Helicobacter pylori* outer membrane protein Q; **HopQAD:** HopQ adhesion domain; **HPSPA:** *H. pylori*-specific peptone agar medium; **HRP:** horseradish peroxidase; **OMP:** outer membrane protein; **rC1ND:** recombinant N-terminal domain of human CEACAM1; **T4SS:** Type IV secretion system; **TMB:** 3,3',5,5'-tetramethylbenzidine

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32 distinct OMP genes have been identified in the *H. pylori* genome, with recent research focusing on some products, including *babA*, *sabA*, *alpA*, *oipA*, and *hopQ*[7-10].

HopQ, an OMP with a molecular weight of approximately 70 kDa, is expressed by *H. pylori* and exhibits specific binding toward CEACAMs, transmembrane receptors on human gastric epithelial cells. Notably, this receptor-ligand interaction distinguishes itself from other Hop family protein mechanisms by operating independently of glycan recognition pathways. Genetic analysis reveals two primary allelic variants of HopQ: Type I, encoded by a 1,926 base pair gene translating to a 642-amino-acid protein, and Type II, derived from a 1,899 base pair gene producing a 633-amino-acid isoform. These variants demonstrate approximately 70% sequence homology at the amino acid level (reviewed in[11]). HopQ has been identified as a crucial component in the process of CagA translocation into host cells via the T4SS. Structurally, its extracellular loops are crucial for mediating adhesion to human CEACAMs, leading to downstream events, including gastric inflammation[12,13]. Initially, integrin receptors on the host side were believed to play a fundamental role in this process[11]. However, recent findings highlight the cell surface CEACAM molecules as the main receptors interacting with *H. pylori* HopQ adhesin. Without these interactions, the process of CagA translocation is either abolished or significantly diminished[14]. It has been demonstrated that HopQ strongly binds to the amino-terminal IgV-like domains of CEACAM 1, 3, 5, and 6, facilitating CagA translocation[15]. This binding is specific to human CEACAM molecules and independent of HopQ glycosylation[15]. The HopQ-CEACAM interaction enables CagA translocation and phosphorylation in the host cell cytoplasm, triggering various pathways by interacting with at least 24 different host proteins in both phosphorylation-dependent and -independent manners[16]. The downstream effects of CagA translocation include cellular proliferation, elongation, disruption of tight junctions, and anti-apoptotic gene regulation, which can lead to more severe outcomes, such as gastric cancer[11].

Multiple antibiotic treatment regimens are employed to eradicate *H. pylori* infection and reduce the incidence of gastric cancer and infection-related morbidities and mortalities[17]. However, increasing drug resistance against *H. pylori* eradication has prompted the World Health Organization to issue a warning[18,19]. The alarming increase in resistance is now extending to younger age groups[20], which is a cause for serious concern. Additionally, patient compliance with multiple drug therapies is less than optimal[21], further intensifying antibiotic-resistant bacteria[22]. Focusing on the HopQ-CEACAM interaction, as the bottleneck in CagA translocation, may yield novel therapeutic agents. In this regard, access to in vitro platforms for studying and manipulating the HopQ-CEACAM interaction, with the aim of identifying novel drugs and potential vaccine candidates, is beneficial. For this purpose, we have developed cost-efficient and easy-to-use antigen and cell-based platforms using recombinant fusion proteins (HopQ-GFP and HopQ-HRP) that interact with the recombinant C1ND, as well as various gastric cell lines (MKN45 and AGS). These platforms facilitate the study of the HopQ-CEACAM interaction and provide means for high-throughput screening of novel inhibition strategies, such as potential drug or vaccine candidates.

MATERIALS AND METHODS

Bacterial culture and DNA extraction

H. pylori GC503 strain was plated onto HPSPA, containing 7% defibrinated sheep blood and incubated in a microaerophilic atmosphere (10% CO_2, 5% O_2, and 85% N_2), at 37 °C for up to 7 days[23]. *H. pylori* growth was identified by colony morphology, Gram staining, urease, oxidase, and catalase tests. Bacterial genomic DNA was then extracted (Favorgen, Iran) and used as the template for PCR amplification.

Cloning, expression, and purification of recombinant proteins

Recombinant HopQ (HopQAD)

PCR amplification of the *H. pylori hopQ* gene fragment encoding amino acids 17-444, corresponding to the HopQ adhesion domain (HopQAD) was performed using primers with NcoI and XhoI sites. The purified amplicon was cloned into pGEM®-T vector and heat-shock transformed into calcium chloride-prepared TOP10F′ *E. coli* cells. Transformants were selected on LB agar with ampicillin (100 µg/ml), tetracycline (15 µg/ml), IPTG (1 mM), and X-gal (40 µg/ml). Blue-white screening, colony PCR, and double digestion were used to confirm cloning accuracy. The *hop*Q fragment was subcloned into pET28a *via* NcoI/XhoI sites, transformed into TOP10F′ cells, and selected on kanamycin (50 µg/ml). Colony PCR, digestion, and sequencing verified the recombinant vector. The *hopQ*-pET28a construct (Fig. 1A) was then transformed into SHuffle® T7 *E. coli* cells. Protein expression was induced at OD_{600} = 0.5 with IPTG (0.5 mM; Sigma, USA) in LB containing kanamycin (50 µg/ml) and spectinomycin (30 µg/ml) at 18 °C for 16 h. Cells were harvested (5300 ×g, 15 min), lysed by sonication (20 × 30 s pulses on ice) in lysis buffer

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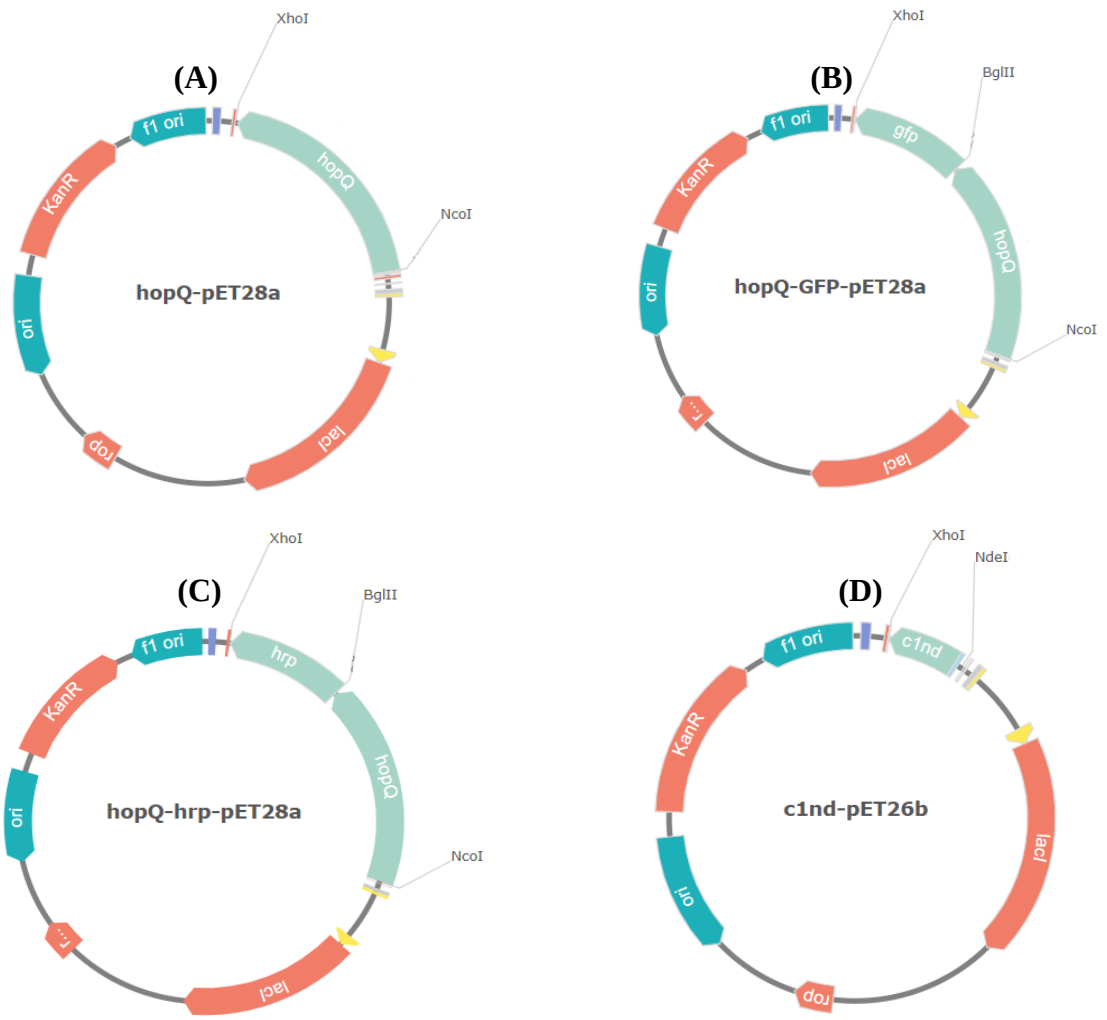

Fig. 1. The schematic views of the various expression vectors used in this study. (A) hopQ-pET28a, (B) hopQ-gfp-pET28a, (C) hopQ-hrp-pET28a, (D) c1nd-pET26b.

(10 mM of imidazole, 300 mM of NaCl, 50 mM of NaH_2PO_4, pH 8.0), and centrifuged (5300 ×g, 20 min). rHopQ protein was purified using Ni-NTA resin (QIAGEN, USA), washed with 30 mM of imidazole buffer, and eluted with 500 mM of imidazole buffer (both with 300 mM of NaCl, 50 mM of NaH_2PO_4, pH 8.0). Protein identity was confirmed by western blotting using anti-His antibody (1:1000, Roche, Germany) and DAB development.

Recombinant HopQ-GFP

PCR amplification of the *hopQ* gene encoding HopQAD was performed using primers with NcoI and BglII sites at the 5′ and 3′ ends, respectively [*hopQAD* (F, R2); Table S1]. The purified PCR product was cloned into pGEM®-T vector and heat-shock transformed into calcium chloride-prepared TOP10F′ *E. coli* competent cells, followed by blue-white screening and confirmation as described for HopQAD. The *gfp* gene fragment was PCR-amplified from the *gfp*-pWS652 plasmid using primers containing BglII and SacI/XhoI sites (Table S1). The purified GFP amplicon was digested and cloned into the hopQ-pGEM vector digested with SacI and XhoI enzymes. The resulting *hopQ-gfp* fusion fragment was subcloned into the pET28a vector between NcoI and XhoI sites, transformed into TOP10F′ cells, and selected on kanamycin (50 µg/ml). Recombinant constructs were confirmed by gene-specific PCR, double digestion, and sequencing. The hopQ-gfp-pET28a plasmid (Fig. 1B) was transformed into SHuffle® T7 competent cells. Protein expression and purification were carried out as described for HopQAD. The identity of the rHopQ-GFP fusion protein was verified by western blotting using anti-His (1:1000) and anti-GFP (1:1000; Santa Cruz, USA) antibodies (Merck, Germany). GFP expression in recombinant *E. coli* was also confirmed 16 hours post-induction by fluorescence microscopy.

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Recombinant HopQ-HRP

PCR amplification of the previously synthesized horseradish peroxidase (*hrp*) gene was performed using gene-specific primers containing BglII and XhoI/SacI sites at the 5′ and 3′ ends, respectively (Table S1). The purified PCR product was cloned into the hopQ-pGEM vector digested with BglII and SacI. The hopQ-hrp-pGEM construct was heat-shock transformed into calcium chloride-prepared TOP10F′ *E. coli* competent cells and plated on LB agar with ampicillin (100 µg/ml), tetracycline (15 µg/ml), IPTG (1 mM), and X-gal (40 µg/ml). Blue-white screening, colony PCR, and double digestion confirmed cloning accuracy. The *hopQ-hrp* fragment was excised using NcoI and XhoI and subcloned into the pET28a vector at the same sites. The recombinant vector was transformed into TOP10F′ cells and selected on kanamycin (50 µg/ml). Confirmation was done by PCR, double digestion, and sequencing. The hopQ-hrp-pET28a plasmid (Fig. 1C) was transformed into SHuffle® T7 competent cells. Protein expression and purification were carried out as described for HopQAD. The identity of rHopQ-HRP was confirmed by western blotting using anti-His tag antibody. Peroxidase activity was verified using the colorimetric substrate TMB (Pishtaz Javid Teb, Iran). As a positive control, HRP conjugation of rHopQ protein (cHopQ-HRP) was performed using an HRP conjugation kit (Abcam, UK).

Recombinant C1ND

PCR amplification of the *c1nd* gene, encoding amino acids 35 to 142 of the CEACAM1 N-domain (C1ND), was performed on genomic DNA from human white blood cells, using gene-specific primers containing NdeI and XhoI sites at the 5′ and 3′ ends, respectively (Table S1). The purified PCR product was cloned into the pGEM®-T vector and heat-shock transformed into calcium chloride-prepared *E. coli* TOP10F′ competent cells. Blue-white screening, colony PCR, and double restriction digestion confirmed cloning accuracy. The *c1nd* fragment was subcloned into the pET26b expression vector at NdeI/XhoI sites and transformed into TOP10F′ cells. Colony PCR, double digestion, and sequencing confirmation, protein expression and purification were performed as described for HopQAD. The identity of rC1ND was confirmed by western blotting using anti-His (1:1000) and anti-CEACAM (1:500, Abcam) antibodies.

SDS-PAGE and western blotting

Recombinant proteins were separated by 12% SDS-PAGE. The resolved protein bands were excised, aligned, and transferred onto nitrocellulose membranes (pore size: 0.45 µm; Bio-Rad, USA) using a semi-dry transfer system (Apelex, France). Membranes were blocked with 3% skim milk at 4 °C overnight. Following blocking, the membranes were incubated with the specific antibody for 4 hours. Subsequently, the strips were washed five times with PBS-T (PBS containing 0.05% Tween 20) and developed using either DAB (Sigma) or ECL (Santa Cruz, USA). Molecular weight estimation of protein bands was performed using either the protein molecular weight markers (Pars Tous, Iran) or pre-stained rainbow markers (SMOBIO, Taiwan).

Flow cytometry

AGS, MKN45, and HEK293 single-cell suspensions were incubated with rHopQ-GFP (2 µg/10^6 cells in 1 ml) at 37 °C, while shaking for 15 minutes. PBS was used as negative control. The cells were washed twice with cold PBS to remove unbound rHopQ-GFP and were then analyzed using a PAS flow cytometer (Partec, Germany). Data were captured and analyzed using Flomax software (Partec, Germany), and the results were displayed as the percentage of positively stained cells, above the gated negative controls. Each sample was run in duplicates, and the mean values ± SD were determined.

Cell culture

AGS, MKN45, and HEK293 cells were grown as a confluent monolayer in DMEM high glucose medium (Bioidea, Iran) supplemented with 10% fetal bovine serum (Thermo Fisher, USA), penicillin (100 U/ml), and streptomycin (100 µg/ml) in 25 cm^2 flasks (SPL, Korea). The flasks were incubated in a 5% CO_2, 100% humidified atmosphere at 37 °C. Once confluency reached 70-80%, the adherent cells were detached using sterile-filtered trypsin-EDTA (0.25%) and centrifuged at 300 ×g for 5 minutes. The cells were resuspended in PBS for further experiments.

Antigen-based ELISA

rC1ND (receptor: 50 µg/ml) was coated onto a 96-well immunoplate (Nunc, MaxiSorb) and incubated at 4 °C overnight. The wells were blocked with 2% BSA (Merck, Germany), for 2 hours at room temperature. Subsequently, the ligand molecules, cHopQ-HRP (50 µg/ml) and serially diluted rHopQ-HRP (0-150 µg/ml), were added to the wells and incubated at room temperature for a further two hours. The wells were washed 3 to 5 times using PBS-0.05% Tween-20. For detection, 1-Step Ultra TMB-ELISA substrate solution (Pishtaz Javid Teb, Iran) was used, and the enzymatic reaction was stopped with 2N HSO_4. Absorbance was measured at 450 nm using an ELISA reader (Biohit, Finland). Each sample was tested in duplicates, and the mean values ± SD were determined.

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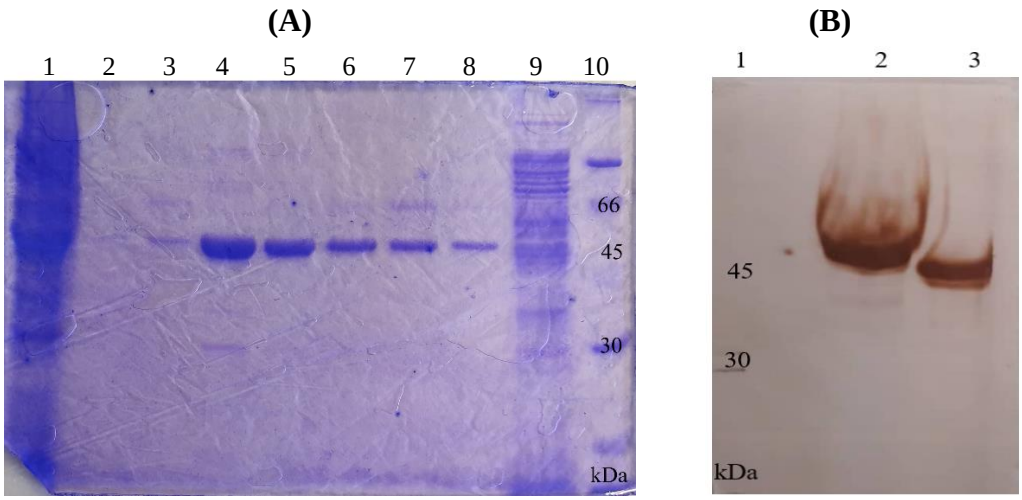

Fig. 2. Expression and purification of the recombinant HopQ protein (~45 kDa). (A) SDS-PAGE: Lane1: initial sample, lane 2-8: elution samples, lane 9: flow through, lane 10: protein marker; (B) western blotting using anti-His tag antibody: lane 1: protein marker, lanes 2 and 3: purified rHopQ proteins.

Cell-based ELISA

AGS cells (5 × 10^4/well) were seeded onto 96-well culture plates (SPL Life Sciences, Korea) and incubated with 5% CO_2 and 100% humidity at 37 °C overnight. The culture medium was discarded, and the cells were fixed using 8% formaldehyde at 4 °C for 30 minutes. Wells were blocked with 3% BSA (Merck) at 4 °C for 2 hours. rHopQ-HRP (20 µg/ml) and serially diluted cHopQ-HRP (0–200 µg/ml) were then added to the wells and incubated on an orbital shaker at 4 °C for 2 hours. After the washing step, 1-Step Ultra TMB-ELISA substrate solution (Pishtaz Javid Teb) was added, and the enzymatic reaction was stopped with 2N HSO_4. Light absorbance was measured at 450 nm using an ELISA reader plate (Biohit). Each sample was tested in duplicates, and the mean values ± SD were determined.

Statistical analysis

Pearson's correlation coefficient (r) was used to assess dose-dependency by examining the correlation between the concentration of rHopQ-HRP and the binding level measured using ELISA assay. Correlation analysis was performed using GraphPad Prism 10.4.1. The significance level was set at $p < 0.05$.

RESULTS

Cell-based CEACAM screening

To quantify CEACAM expression levels in different cell lines, we first cloned the *hopQAD* gene fragment into the pET28a vector (Fig. 1A). The gene fragment was sequenced and deposited in GenBank (MZ541988.1). The recombinant HopQ adhesion domain was expressed in a soluble format, which was then purified (Fig. 2A). The identity of the purified rHopQ was confirmed *via* western blotting (Fig. 2B). After successfully purifying the recombinant HopQ protein, with a molecular weight of approximately ~45 kDa, we cloned the *gfp*-encoding gene fragment into the created hopQ-pET28a expression vector (Fig. 1B). Confirmation of the GFP moiety's expression in the fusion protein was achieved through fluorescent microscopy observations, as illustrated in Figure 3. We then purified the recombinant HopQ-GFP fusion protein, with an estimated molecular weight of ~73 kDa (Fig. 4A), and confirmed its identity using both anti-His tag (Fig. 4B) and anti-GFP (Fig. 4C) antibodies. To evaluate its capacity in screening CEACAM molecules on the surfaces of three different cell lines, we performed flow cytometry analysis on AGS (Fig. S1), MKN45 (Fig. S2), and HEK293 (Fig. S3) cells using rHopQ-GFP. As shown in Figure 5, this analysis revealed that MKN45 cells displayed the highest level of CEACAM expression (97%), followed by AGS cells with less than 10% expression, and HEK293 cells exhibited minimal binding, reflecting their actual low CEACAM expression levels.

Antigen based ELISA platform

To evaluate the interaction between HopQ and CEACAM as ligand and receptor, using both antigen-based and cell-based ELISA platforms, we conducted a series of experiments. First, we cloned the *hrp* gene fragment downstream of the *hopQ* encoding gene fragment in the pET28a vector (Fig. 1C). This setup allowed us to produce a fusion protein that included both

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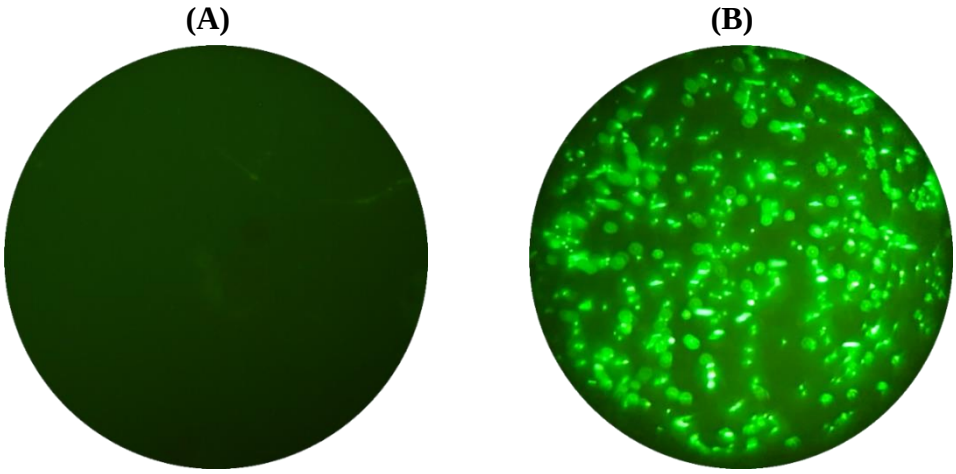

Fig. 3. HopQ-GFP expressing *E. coli* cells under fluorescent microscopy. (A) Parent strain without GFP expression (negative control); (B) recombinant strain. (100 ×).

the HopQ and HRP components with an estimated molecular weight of 75 kDa (Fig. 6A), which was confirmed for identity by the anti-His antibody (Fig. 6B). For the receptor in our antigen-based ELISA, we cloned the *c1nd* gene, encoding for the C1ND segment, into the pET-26b vector (Fig. 1D), expressed and purified the rC1ND protein, with an approximate molecular weight of ~10 kDa (Fig. 7A), and confirmed its identity using both anti-His tag (Fig. 7B) and anti-CEACAM (Fig. 7C) antibodies. Upon conducting the antigen-based ELISA assay, we observed a dose-dependent interaction between the rC1ND and the rHopQ-HRP fusion proteins (Fig. 8A). As the concentration of rHopQ-HRP increased, its interaction with rC1ND also increased, demonstrating a direct correlation between the ligand concentration and receptor binding (r = 0.95, p = 0.014).

Cell-based ELISA platform

To further evaluate the interaction between HopQ and CEACAM, we performed a cell-based ELISA assay using the AGS cell line. This cell line is commonly employed in the studies related to the pathogenesis of *H. pylori* and is known for its moderate level of CEACAM expression. In the cell-based ELISA, we aimed to verify the ligand-receptor binding observed in the antigen-based ELISA. Consistent with the results from the antigen-based ELISA, we observed a dose-dependent ligand-receptor binding in the cell-based ELISA (Fig. 8B, r = 0.998, p = 0.039). As the concentration of rHopQ-HRP protein increased, the binding to CEACAM molecules on the surface of AGS cells also increased, demonstrating a similar pattern of interaction.

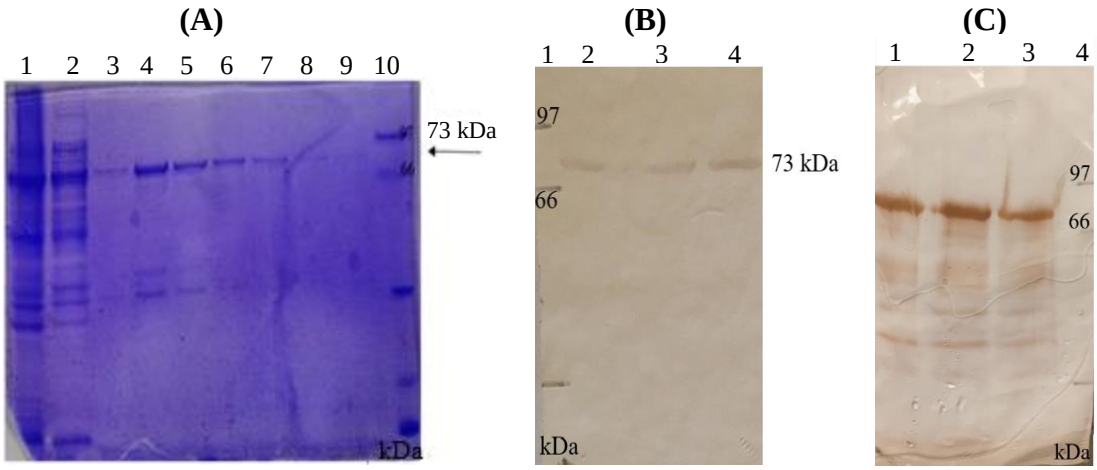

Fig. 4. Expression and purification of recombinant HopQ-GFP fusion protein (~73 kDa). (A) SDS-PAGE: lane 1: initial sample, lane 2: flow through, lanes 3-9: eluted protein, lane10: protein marker; (B) western blotting using anti-His antibody: lane 1: protein marker, lanes 2-4: purified rHopQ-GFP protein; (C) western blotting using anti-GFP antibody: lane 1-3: purified rHopQ-GFP protein, lane 4: protein marker.

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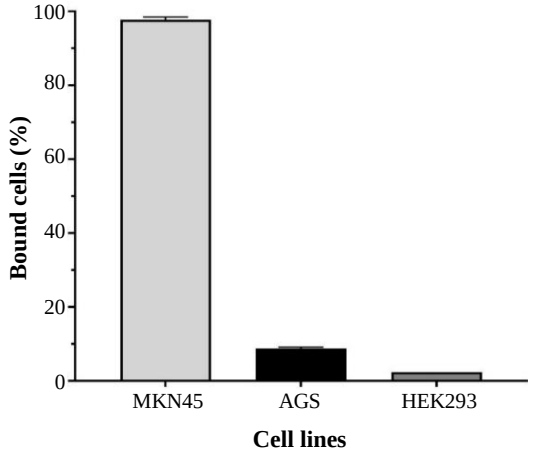

Fig. 5. Detection of CEACAM expression on the surface of different cell lines by flow cytometry, using rHopQ-GFP protein. Mean values ± SD are presented.

DISCUSSION

Infecting half of the world's population, *H. pylori* is classified as a group one carcinogen by the International Agency for Research on Cancer (IARC)[19]. This bacterium is identified as the most infectious agent causing cancer[24]. The oncogenicity of *H. pylori* is primarily attributed to its CagA bacterial oncoprotein. *H. pylori* HopQ is believed to play a crucial role in CagA translocation, leading to its phosphorylation, cell elongation, and IL-8 production[14]. Thus, focusing on HopQ and HopQ-CEACAM interaction as a potential therapeutic/preventative target has become of interest. Furthermore, antibiotic overuse results in increasing antibiotic resistance, which is the major cause of eradication failure in the *H. pylori* treatment process[25]. New drugs and eradication regimens can help us to overcome this infection. Javaheri and colleagues[8] have demonstrated that pre-incubating AGS cells with anti-CEACAM1 or anti-HopQ antibodies significantly reduce CagA translocation during infection. Similarly, introducing HopQID, a peptide derived from HopQ, prior to infection effectively decreased both CagA translocation and the elongation phenotype of infected cells in a dose-dependent and competitive manner[8]. Further advancing this concept, Hanafiah *et al.*[26] conducted molecular docking studies to explore the interactions between AMPs and *H. pylori* proteins, including HopQ. Their findings revealed that type I HopQ exhibited the strongest binding affinity with EcAMP1, an AMP, as evidenced by a binding affinity of -13.8 kcal/mol and the formation of 28 hydrogen bonds. Molecular dynamics simulations corroborated the stability of this interaction, suggesting its potential to inhibit HopQ-mediated pathogenic mechanisms[26].

Inhibiting or reducing bacterial pathogenesis as an alternative to antibiotics, offers benefits, such as avoiding the development of antibiotic resistance and preserving the natural integrity of microbiome[27]. Building on this concept, Bendary *et al.*[28] evaluated the effect of curcumin supplementation on downregulating adhesin expression, particularly HopQ, thereby reducing adhesin pathogenicity and enhancing *H. pylori* eradication in a mouse model. Hollandworth *et al.*[29] demonstrated that HopQ-IR800, a fluorescently labeled HopQ protein, can specifically bind to CEACAM

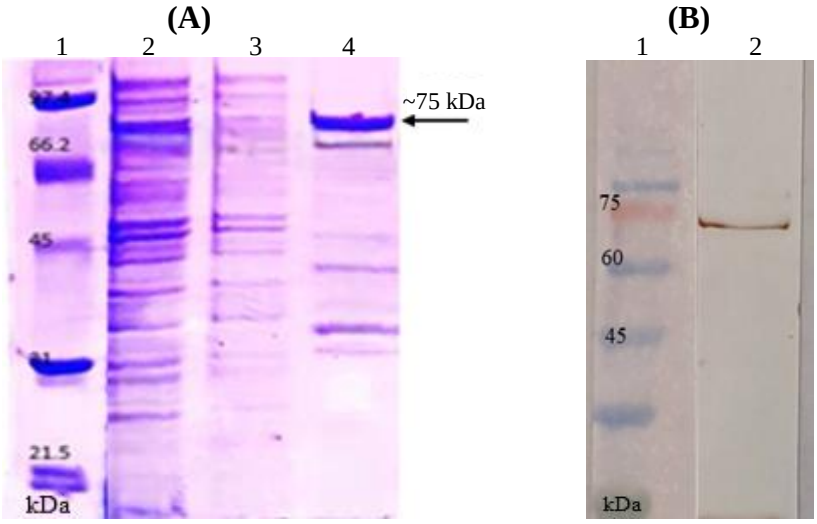

Fig. 6. Expression and purification of the recombinant HopQ-HRP (~75 kDa). (A) SDS-PAGE: lane 1: protein marker, lane 2: Initial sample, lane 3: flow through, lane 4: purified rHopQ-HRP; (B) western blotting using anti-His antibody: lane 1: protein marker, lane 2: purified rHopQ-HRP.

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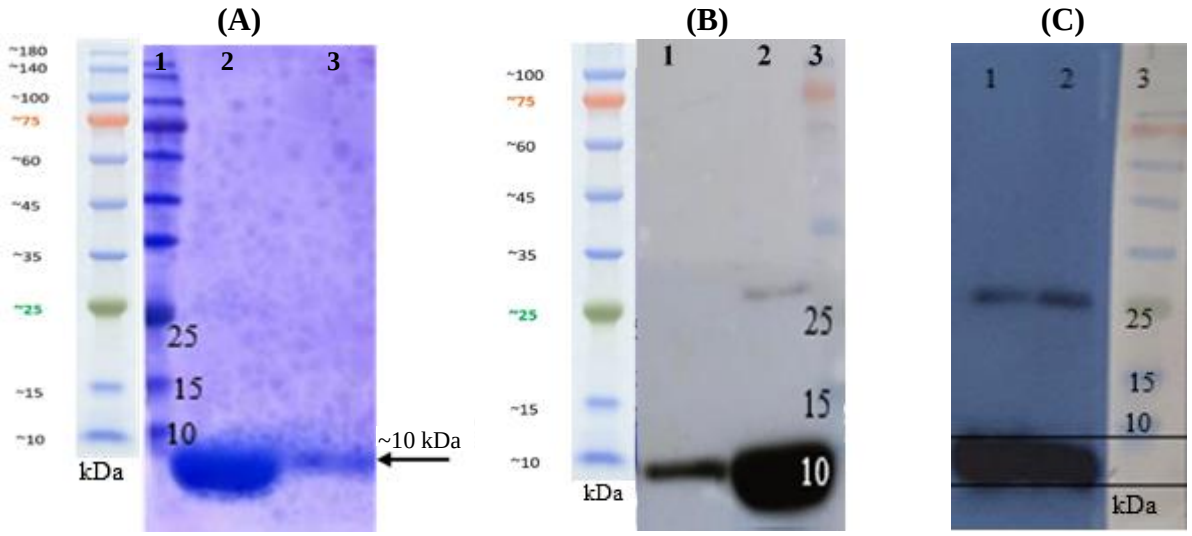

Fig. 7. Expression and purification of the rC1ND (~10 kDa). (A) SDS-PAGE: lane 1: protein marker, lanes 2 and 3: purified protein samples; (B) western blotting using anti-His antibody: lane 1: protein marker, lanes 2 and 3: purified r-C1ND, (C) western blotting using anti-CEACAM antibody: lanes 1 and 2: purified r-C1ND.

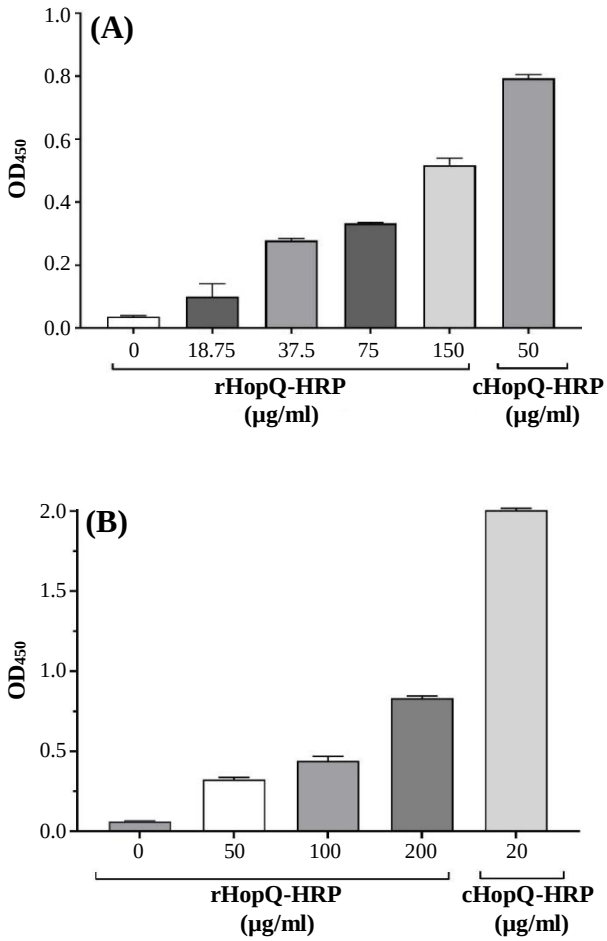

Fig. 8. Quantification of rHopQ-HRP (ligand) binding to rC1ND (receptor) in an antigen-based (A) and to CEACAM molecules (receptor) on AGS cells in a cell-based ELISA (B). cHopQ-HRP was used as a positive control. Mean values ± SD of duplicates are presented.

molecules overexpressed in colon tumors in mice, enabling clear visualization of tumor margins during fluorescence-guided surgery. This approach shows promise for improving surgical precision in oncology. Similarly, our rHopQ-GFP fusion protein can detect CEACAM expression in various cell lines and holds potential for *in vivo* applications to identify CEACAM-overexpressing cells.

Access to *H. pylori* recombinant proteins enables assessment of host antibody responses. Song *et al.*[30] found that anti-HopQ antibodies significantly increase the risk of intestinal metaplasia and show a synergistic effect with other antigens like CagA, Omp18, and TlpB. Additionally, Jaradat and colleagues[31] developed a sensitive biosensor using anti-HopQ antibodies to detect HopQ as an early biomarker of *H. pylori*, useful for screening contaminated resources such as drinking water. To further investigate the HopQ-CEACAM interaction and identify therapeutic candidates, we have designed cost-effective and straightforward platforms for both antigen and cell-based assays. These platforms were developed to simulate the interaction, enabling the evaluation of the specificity and dose dependency of potential inhibitors. For the antigen-based simulation, we produced the ligand (HopQ-HRP) and the receptor (C1ND) in recombinant forms. To validate the peroxidase activity of rHopQ-HRP and its reliability, we used conjugated HopQ-HRP as a positive control. Results demonstrated that the interaction between rHopQ-HRP and rC1ND occurred in a dose-dependent manner. While antigen-based ELISA is a straightforward and efficient method to quantify interactions, it has limitations in replicating the complexity of the cellular environment. For instance,

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multiple CEACAM molecules other than CEACAM1 are present on the epithelial cell membrane, which could influence the interaction. To overcome these limitations, we developed a cell-based ELISA platform that more accurately reflects the cellular environment. This method combines immunocytochemistry and quantitative analysis of interactions between cell surface-expressed receptors and their corresponding ligands. For the first layer of the assay, we selected two gastric cancer cell lines, AGS and MKN45, both commonly used in *H. pylori* research. AGS cells are adherent, while MKN45 cells are predominantly suspension-based. Our cell screening using the rHopQ-GFP molecule in flow cytometry to measure CEACAM expression levels, demonstrated that MKN45 cells exhibit higher levels of CEACAM compared to AGS cells. However, due to their adherent nature, AGS cells were chosen for the cell-based ELISA platform. CEACAM expression on AGS cells was confirmed by flow cytometry and corroborated by previous studies[32]. Results from the cell-based ELISA showed that rHopQ-HRP binds to CEACAM molecules on the AGS cell surface in a dose-dependent manner. This interaction is thought to resemble the natural conditions under which *H. pylori* adheres to gastric epithelial cells and may offer a useful platform for screening therapeutic candidates. However, it should be noted that this assay requires further validation through complementary tests and is intended primarily as an initial step for evaluating potential candidates.

CONCLUSION

The HopQ-CEACAM interaction presents a promising target for therapeutic intervention, given its crucial role in the pathogenesis of *H. pylori*. The assay platforms developed and presented here, enable detailed investigation of the HopQ-CEACAM interaction and further exploration of small molecule inhibitors, peptides, or antibodies targeting this interaction in order to develop effective therapies for the management of *H. pylori* infection. Such advancements could significantly improve treatment outcomes, reduce antibiotic resistance, and enhance overall patient compliance. Additionally, innovative approaches like fluorescence-guided surgery and advanced diagnostic tools underscore the broader implications of HopQ research in medical science.

DECLARATIONS

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Ethical approval

All the experimental procedures in this study were approved by the Research Ethics Committee of Pasteur Institute of Iran, Tehran, Iran (ethical code: IR.PII.REC.1400.079/IR.PII.REC.1399.082).

Consent to participate

Not applicable.

Consent for publication

All authors reviewed the results and approved the final version of the manuscript.

Authors' contributions

NSh: performed the HopQ-related experiments and wrote the first draft of the manuscript, ME: assisted in carrying out the experiments, KA: cloned, expressed and purified rHopQ-HRP; NAH: cloned, expressed and purified rC1ND, MF: cloned, expressed and purified rHopQ-GFP, NS: cloned, expressed and purified rHopQ; YT, FK, and EM: provided expert guidance; MM: designed and supervised the study, revised and approved the final draft.

Data availability

All relevant data can be found within the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Supplementary information

The online version contains supplementary material.

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