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Regulation of cagA-*Helicobacter* on gastric PIM2 expression in gastric cancer

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Abstract.
BACKGROUND: The association between infection with *cagA*-positive *H. pylori* and an elevated susceptibility to gastric cancer has been firmly established. PIM2 is known to be overexpressed in various types of cancers; however, the specific mechanism by which *cagA* influences the regulation of PIM2 expression in gastric cancer remains unidentified at present.
MATERIALS AND METHODS: A mutant NCTC11637ΔcagA strain of *H. pylori* and the eukaryotic expression vector pcDNA-*cagA* were constructed for evaluating PIM2 expression levels in gastric cancer cells (HGC27, SGC7901, and AG) co-cultured with the NCTC11637 and NCTC11637ΔcagA strain, as well as pcDNA-*cagA* and the empty vector pcDNA3.1 (+).
RESULTS: Co-culturing gastric cancer cells with NCTC11637 significantly increased PIM2 expression levels ($P < 0.001$) compared to the negative control group. Additionally, the expression of PIM2 in cells co-cultured with NCTC11637 was higher than that co-cultured with NCTC11637ΔcagA ($P < 0.001$). Furthermore, successful construction of the eukaryotic expression vector pcDNA-*cagA* resulted in a significant increase in PIM2 mRNA expression levels after its transfection into gastric cancer cells compared to the control group after 48 hours.
CONCLUSIONS: The findings indicate that H. *pylori*/*cagA* A could be one of the key factors in regulating PIM2 expression levels, potentially influencing the progression of *H. pylori*-related Gastric Cancer.

Keywords: Gastric cancer, PIM2, *Helicobacter pylori*, CagA, regulation

1. Introduction

Gastric cancer (GC) remains a significant public health issue worldwide, despite recent declines in incidence and mortality rates [1]. Risk factors for gastric cancer are older age, low socio-economic status, cigarette smoking, alcohol consumption, familial predisposition, previous gastric surgery, pernicious anaemia, and living in a population at high risk, and *Helicobacter pylori* (*H. pylori*) infection is the most well described risk factor for gastric cancer [2]. Chronic inflammation and the pathogenesis of gastric cancer (GC) are posited to be precipitated by the activation of the nuclear transcription factor kappa B (NFκB) induced by *H. pylori,* subsequently resulting in the upregulation of interleukin-8 (IL-8) [3,4]. Specifically, within gastric epithelial cells, the cytotoxic-associated gene A protein (CagA), a predominant virulence factor of *H. pylor*i, is responsible for the activation of NF-κB through the canonical pathway [5,6]. The subsequent increase in IL-8, mediated by NF-κB, is implicated in the perpet-

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uation of chronic gastritis and the propagation of inflammatory responses [4,5]. Our preceding studies have uncovered that *H. pylori* infection and the CagA protein may exert effects on gastric epithelial cells through the modulation of α-enolase (ENO1) [7].

The proviral integration sites for Moloney murine leukemia virus (PIMs) represent a group of serine/threonine kinases that are overexpressed in various hematological malignancies and solid tumors, exerting regulatory effects on cell proliferation and apoptosis [8]. The PIM family comprises three isoforms (PIM1, PIM2, and PIM3), which exhibit high conservation across evolutionary lines [8]. While PIM1 and PIM3 are predominantly expressed in hematological and solid tumors, respectively, PIM2 is primarily associated with leukemia [9]. Functionally, PIM2 plays a role in cell apoptosis, proliferation, and differentiation [10], and has been identified as one of the downstream targets of NFκB [11]. However, the specific involvement of PIM2 in *H. pylori*-related GC development remains to be elucidated.

In this study, we explored the impact of *H. pylori*/cagA on PIM2 expression in gastric cancer cells, using a cagA knockout mutant and a cagA expression vector, uncovering a new mechanism of *H. pylori*-induced gastric pathogenesis through PIM2 regulation.

2. Materials and methods

2.1. Reagents and cell lines

ClonExpress II One Step Cloning Kit, ClonExpress MultiS One Step Cloning Kit, ChamQ Universal SYBR qPCR Master Mix and HiScript® II Q RT SuperMix for qPCR (+gDNA wiper) were supplied by Vazyme Biotechnology Co., Ltd. (Nanjing, China). Lipofectamine 2000® and TRIzol were obtained from Invitrogen (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Takara Biotechnology Co., Ltd. (Dalian, China) provided PrimeSTA® GXL DNA Polymerase and *E. coli* DHα Competent Cells. Mouse anti-CagA antibody (cat. no.sc-28368) was purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Anti-beta actin mouse monoclonal antibody (cat. no. A01010) and Goat Anti-Mouse IgG-HRP (cat.no.SA00001-1) were supplied by Abbkine Scientific Co., Ltd. (Wuhan, China) and Proteintech Group, Inc. (Rosemont, IL, USA), respectively. In this study, the GC cell lines, HGC27 (PRIMARY Biological, Shanghai, China), SGC7901 and AGS (Procell Life, Wuhan, China), were utilized. The HGC27 and SGC7901 cells were maintained in RPMI-1640 medium (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (Cegrogen Biotech GmbH, Germany). The AGS cells were cultured in F12 medium (HyClone, Logan, UT, USA).

2.2. Construction of H. pylori cagA knocked-out strain

The upstream and downstream homologous fragments of *cagA* derived from *H. pylori* NCTC11637, as well as chloramphenicol resistance gene (cmR) originating from the plasmid pNZ8110, were successfully amplified by PCR. The primers were delineated as follows: P1-F1 sense, 5-CGAGGTCGACGGTATCGATA AGCTTTGCTCGGCAACTGACATCA-3, P2-F1 antisense, 5-ATCAATT TTATTAAAGTTCATTGTTTCTC CTTACTATACC-3; P3-F2 sense, 5-CCTAATGACT GGCTTTTATAAAGGATTAAGGAA TACCAAAAAC G-3, P4-F2 anti-sense, 5-GCGGT GGCGGCCGCTC TAGATTCCACTTCTGATCGCAAGC-3; P5-cmR sense, 5-GGTATAG TAAGGAGAAACAATGAACTTT AATAAAATTGAT-3, P6-cmR anti-sense, 5-CGTTTTT GGTATTCCTTAATCCTTTATAAAGCCAGTCATT AGG-3. PCR conditions included pre-denaturation at 95°C for 5 min followed by 35 cycles consisting of denaturation at 94°C for sixty seconds, annealing at 57°C for fifty seconds, and extension at 72°C also lasting sixty seconds; concluding with a final ten-minute extension step set again to 72°C. The Clon Express MultiS One Step Cloning Kit facilitated insertion of fragments into plasmid pBluescript IISK (-). The recombinant plasmid underwent transfer into NCTC11637 by electroporation, and subsequently, the mutant strain was selected on a chloramphenicol agar plate. Identification of the mutant strain NCTC1163Δ*cagA* entailed PCR, sequencing and Western blot to identify.

2.3. Construction of cagA eukaryotic expression vector

The *cagA* gene fragment was gained via PCR from the genomic DNA of NCTC11637. Then, the plasmid pcDNA3.1 (+) along with seamless cloning technique were utilized to construct the eukaryotic expression vector pcDNA-*cagA*. The primer sequences were showed as follows: P7-CagA, 5-CAGTGTGGTGGAATTCGCC AC ATGACTAACGAAACCATTGACC-3; P8-CagA, 5-AACGGGC CCTCTAGACTCGAGTTAAGATTTT TGGAAA CCACCTTTT-3.

2.4. Co-culture of H. pylori with GC cells

Initially, *H. pylori* NCTC11637 was co-cultured with the GC cells, namely HGC27, SGC7901 and AGS, un-

der multiplicity of infection (MOI) ratios of 50:1, 100:1 and 150:1, respectively. Subsequently, the expression levels of PIM2 mRNA within these cells were quantitatively assayed at 24-hour of co-culture initiation. Subsequently, employing NCTC11637 strain as a reference control, the NCTC1163Δ*cagA* was co-cultured with the GC cells for 24 hours at an MOI of 150:1. The expression of PIM2 mRNA was then precisely quantified using qPCR.

2.5. Cellular transfection

Upon successful identification and confirmation of the recombinant plasmid construct, the gastric cancer cell lines HGC27, SGC7901 and AGS cells were individually subjected to transfection with pcDNA-*cagA*. Concurrently, a control group was established using the empty vector pcDNA3.1 (+). Post-transfection, the cells were incubated for a period of 48 hours, following which the expression levels of PIM2 mRNA were evaluated employing quantitative polymerase chain reaction (qPCR) methodology.

2.6. Reverse transcription-quantitative PCR (RT-qPCR)

Total RNA was extracted using TRIzol reagent, strictly according to the manufacturer's protocols provided by the manufacturer. Subsequently, a 20 μl reaction system was employed for the reverse transcription process, utilizing HiScript® II Q RT SuperMix for qPCR kit. The qPCR reaction was performed using ChamQ Universal SYBR qPCR Master Mix, according to the manufacturer's protocol. The primer sequences were as follows: CagA forward, 5-ACGC TCTGTCTTCTGTGCTAATGG-3; CagA reverse, 5-AATCATGCCTAGCTTCAGGACCAC-3; PIM2 forward, 5-GCCATCCAGC ACTGCCATTCC-3; PIM2 reverse, 5-ACAGCCACGGCGTAGGTCTATC-3; GAP DH forward, 5-ACCACAGTCCATGCCATCAC-3; GA PDH reverse, 5-TCCACCACCCTG TTGCTG TA-3. The qPCR conditions: Pre- -denaturation at 95°C for 30 s; 40 cycles of 95°C for 10 s and 60°C for 30 s. The relative expression of CagA and PIM2 was normalized to GAPDH, and the expression levels were calculated using the 2-ΔΔCq method.

2.7. Western blot

Total protein was extracted from cells employing RIPA Lysis buffer (ComWin Biotech Co., Ltd., Beijing, China), which was supplemented with a 1% protease inhibitor cocktail (ComWin Biotech Co., Ltd., Beijing, China). The protein concentration was quantified using the BCA Protein assay kit (Thermo Fisher Scientific, Inc.). Equivalent quantities of protein extracts (50 μg) were resolved through SDS-PAGE conducted on a 10% gel. The resolved proteins were then transferred onto a polyvinylidene fluoride membrane, which was subsequently blocked with 5% w/v non-fat dried milk dissolved in TBS with Tween-20 (TBS-T, 0.1% Tween-20, pH 8.3) for 1 h at room temperature, and incubated with primary antibodies at 4°C overnight. The following primary antibodies were used: antiβ-actin (1:1000) and anti-CagA (1:1000). Following washing with TBS-T, the membranes were incubated with HRP-labeled anti-rabbit or mouse IgG secondary antibody (both 1:3000) for 1 h at room temperature. Bands were visualized using an enhanced chemiluminescence kit (ComWin Biotech Co., Ltd., Beijing, China) and Amersham Imager 600.

2.8. Statistical analysis

All data were analyzed utilizing the SPSS software (version 17.0; SPSS, Inc., Chicago, IL, USA). Quantitative data were presented as the mean ± standard deviation. Comparisons of groups were carried out using Student's t-test and one-way analysis of variance (ANOVA). To further elucidate these differences, the Bonferroni test was applied for multiple comparison of groups. $P < 0.05$ was considered as statistically significant difference.

3. Results

3.1. The expression of PIM2 at different multiplicity of infection (MOI) levels with H. pylori

The Fig. 1 demonstrated that the expression levels of PIM2 at varying multiplicities of infection (MOI) with *H. pylori*. The experimental group with an MOI of 150 exhibited a significant upregulation of PIM2 mRNA expression compared to the control group across three gastric cancer cell lines, HGC27, SGC7901 and AGS ($P < 0.05$). In addition, the PIM2 mRNA expression in the experimental group at MOI of 10 was significantly elevated relative to the control group in HGC27and SGC7901 gastric cancerous cell lines ($P < 0.05$). Conversely, no statistically significant difference in PIM2 mRNA expression was observed at an MOI of 50:1 in

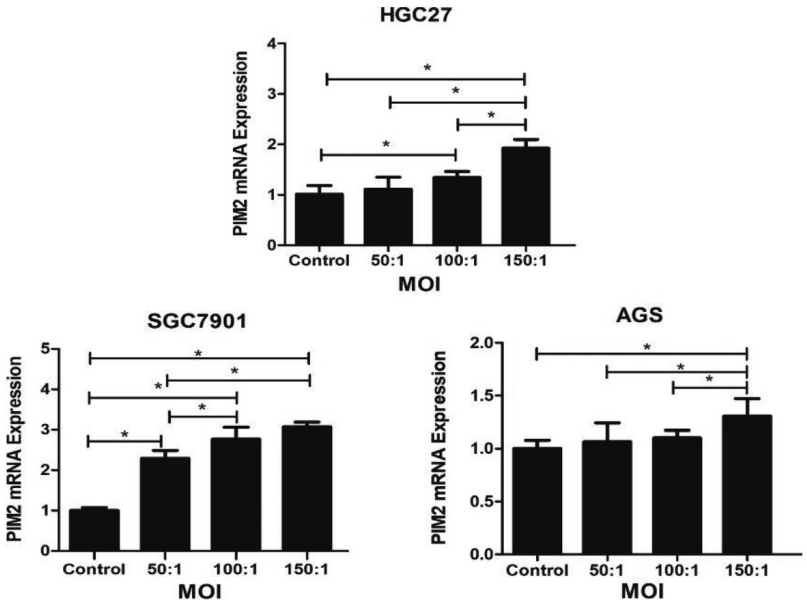

Fig. 1. Expression of PIM2 mRNA in GC cells infected with *H. pylori* at a range of MOIs. ($^*P < 0.05$).

HGC27and AGS gastric cancer cells. Additionally, our findings indicate an MOI-dependent increase in PIM2 transcription, with the most pronounced elevation occurring at an MOI of 150, in the infected GC cells, as compared to the control groups.

We subsequently investigated the expression levels of PIM2 mRNA in *H. pylori* GC27, SGC7901, and AGS cells after infection with *H. pylori* strain NCTC11637 at an MOI of 150:1 (Fig. 2). The results revealed a significant upregulation of PIM2 mRNA expression in HGC27 cells at 12 h ($P = 0.001$) and 24 h ($P = 0.001$) post-infection with NCTC11637, whereas no significant difference in PIM2 mRNA expression was observed at 6 h ($P = 0.117$). Similarly, infection of SGC7901 cells with NCTC11637 for 6 h ($P < 0.001$), 12 h ($P = 0.015$), and 24 h ($P < 0.001$) resulted in a significant increase in PIM2 mRNA expression. Furthermore, NCTC11637 infection of AGS cells for 6 h ($P < 0.001$), 12 h ($P < 0.001$), and 24 h ($P < 0.001$) also led to a marked elevation in PIM2 mRNA expression compared to the control group.

3.2. The expression of PIM2 infected with NCTC11637 and NCTC11637 ΔcagA H. pylori Strains

We successfully constructed the *H. pylori* Δ*cagA* strain, which was molecularly identified via PCR (Fig. S1) and confirmed by Western blot analysis (Fig. S2). Subsequently, the GC cells were co-cultured with *H. pylori* NCTC11637 (*cagA*+) and NCTC11637Δ*cagA* mutants for 24 hours. Compared to the uninfected controls, both the *cagA*+ *H. pylori* and the Δ*cagA H. pylori* infection resulted in elevated PIM2 mRNA expression in three cancerous cells ($P < 0.05$) (Fig. 3). However, the expression level of PIM2 mRNA was significantly higher in the cells infected with the *cagA*+ strain than those with the Δ*cagA* mutants ($P < 0.05$).

3.3. The expression of PIM2 infected with the eukaryotic expression vector construction of NCTC11637 cagA

The eukaryotic expression vector pcDNA-*cagA* was successfully constructed as confirmed by PCR (Fig. S3). Subsequent transfection of HGC27, SGC7901 and AGS cells with pcDNA-*cagA* showed increased expression of cagA gene as detected by qPCR and Western blot analysis, confirming successful transfection into cells (Fig. 4). The expression levels of PIM2 mRNA in HGC27, SGC7901 and AGS cells transfected with pcDNA-*cagA* for 48 hours, were significantly higher than control cells treated with pcDNA3.1(+) ($P < 0.05$) (Fig. 5).

4. Discussion

PIM2 kinase plays an important role as an oncogene in multiple cancers, such as leukemia, liver, lung,

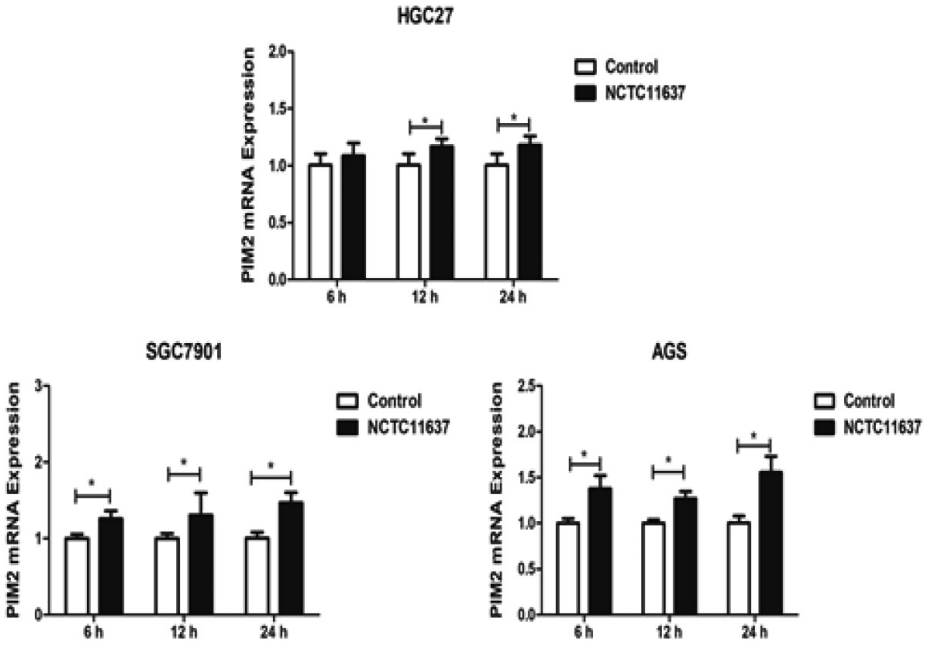

Fig. 2. The expression of PIM2 mRNA in *H. pylori* NCTC11637-infected HGC27, SGC7901, and AGS cells at 6 h, 12 h, and 24 h post-infection ($^*P < 0.05$).

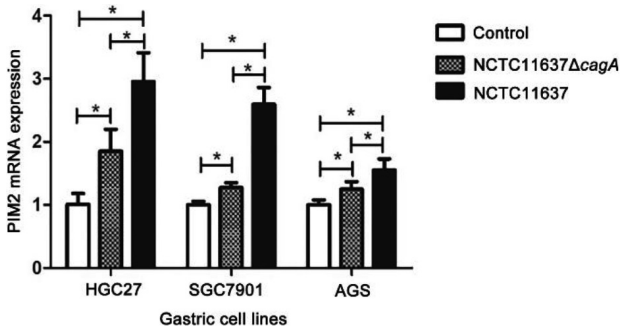

Fig. 3. Expression of PIM2 mRNA in the GC cells co-cultured with *H. pylori* (*cagA*+) and *H. pylori* (Δ*cagA*), respectively ($^*P < 0.05$).

myeloma, prostate and breast cancers. PIM2 is largely expressed in both leukemia and solid tumors, and it promotes the transcriptional activation of genes involved in cell survival, cell proliferation, and cell-cycle progression [12]. This study revealed that the MOI and duration of *H. pylori* infection upregulated the expression of PIM2 in gastric cancer cells. Furthermore, our findings provided additional evidence for the regulatory effect of *cagA* on PIM2 expression in gastric cancer epithelial cells by demonstrating an increase in PIM2 expression following transfection with *cagA*.

H. pylori colonizes the human gastric mucosa and significantly increases the risk of GC [2]. *H. pylori* can activate NFκB and enhance the production of pro-inflammatory cytokines. NFκ involved not only in the inflammatory response but also in the regulation of immune response, cell proliferation, differentiation and apoptosis [5,6]. Furthermore, *H. pylori* infection can promote the expression of programmed death ligand 1 (PD-L1) in gastric epithelial cells through the JAK/STAT transcription signaling pathway, thereby, suppressing immune surveillance, and facilitating gastric carcinogenesis [14].

The PIM2 gene is recognized as an oncogene, which plays an essential role in the signal transduction process related to cell proliferation, differentiation, apoptosis, migration, survival and metabolism. It is also transcriptionally regulated by the JAK/STAT and NFκ signaling pathways [11,13,15]. Our study observed a correlation between *H. pylori* infection and PIM2 expression in

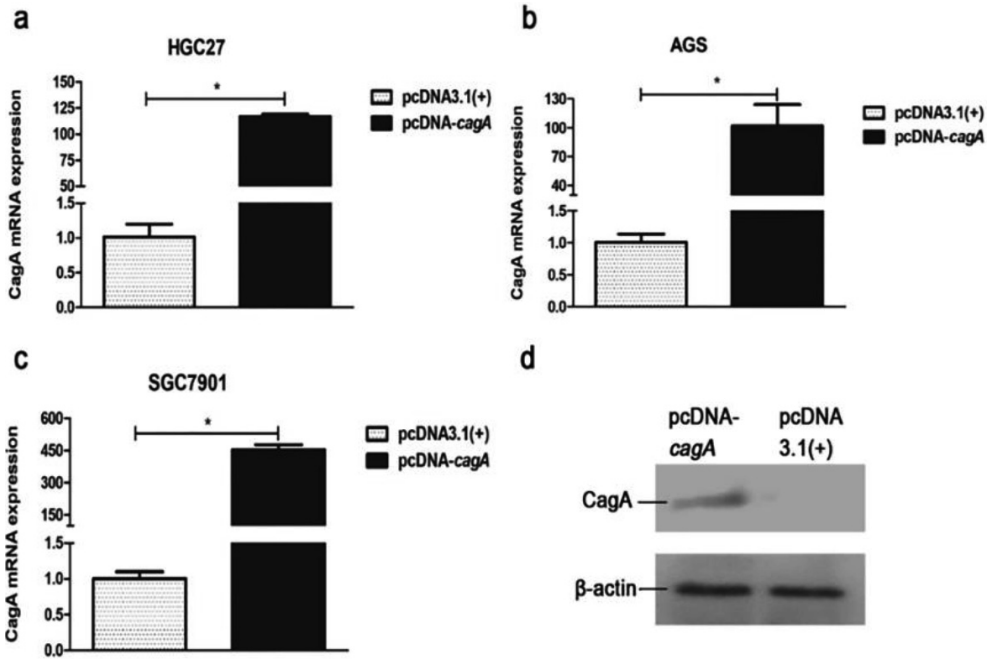

Fig. 4. Expression of *cagA* gene in the GC cells transfected with pcDNA-*cagA* plasmid. (a) (b) (c) CagA mRNA expression in the GC cells, HGC27, AGS and SGC7901, transfected with pcDNA-*cagA* and pcDNA3.1(+), respectively, for 48 h; (d) Expression of CagA in the SGC7901 cells transfected with pcDNA-*cagA* for 48 h ($^*P < 0.05$).

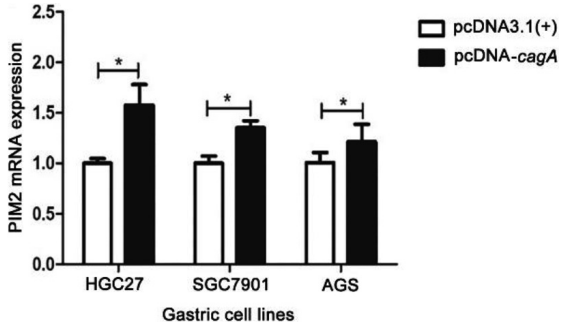

Fig. 5. PIM2 mRNA expression in the GC cells transfected with pcDNA-*cagA* plasmid. ($^*P < 0.05$).

GC cells, potentially mediated by the JAK/STAT and/or NFκB signaling pathways. Furthermore, our data indicated that the PIM2 expression levels proportionally with the rising MOIs of *H. pylori*-GC cell coculture. This finding supports the notion that antibiotic therapies or vaccination may mitigate *H. pylori*-induced lesions primarily by reducing gastric bacterial colonization, which holds significant implication for clinical practices.

The CagA is s one of the most widely studied *H. pylori* virulence factor. Previous studies have demonstrated that infection with *H. pylori cagA* (+) strains significantly increased the risk of gastric carcinoma [16]. The present study provided evidence of the significant impact of CagA on PIM2 expression in GC cells through co-culture experiments involving *H. pylori* (*cagA*+/Δ*cagA*) and GC cells, as well as cellular transfection. The mechanism and significance of the CagA effect on PIM2 need to be further investigated.

As documented, CagA can be injected into target cells. Upon once translocation into the host cytoplasm, CagA may bind to the inner surface of the cell membrane and interact with several host proteins, thereby activating downstream signaling pathways such as NFκB and β-catenin pathway [13,17]. Besides, CagA can modulate the activity of kinase/mitogen-activated protein kinase (ERK/MAPK), resulting to abnormal proliferation and migration of gastric epithelial cells [18].

Furthermore, CagA has been reported to induce the production of pro-inflammatory cytokines and anti-apoptotic proteins by activating STAT3, as well as to activate transcription factors via the JAK/STAT signaling pathway in response to inflammation [19,20]. In vitro infection of cells with *H. pylori* (*cagA*+) strains can result in formation of an elongated cell shape, known as "hummingbird phenotype" [21]. This morphological alteration can promote the occurrence of epithelial-mesenchymal transition (EMT) [22]. These findings indicated that CagA may have multiple functions in the development of *H. pylori*-associated. The present study elucidated the role of CagA in modulating PIM2 production, thereby providing novel insights into the role of *H. pylori* in gastric pathogenesis.

The CagA-induced regulation of PIM2 overexpression may represent a critical mechanism underlying the development of GC. Santio et al. demonstrated that in hematopoietic cells, cytokines, hormones or antigens predominantly upregulated PIM2 gene expression mainly via the JAK/STAT signaling pathway [23]. Previous studies has established that STAT protein are regulators of EMT [24]. In breast cancer cells, PIM2 indirectly activates STAT3 protein by upregulating the production of cytokines IL-α and IL-.The activated STAT3, in turn, regulates the transcription of PIM2, thereby establishing a positive feedback loop that continuously activates STAT3 and subsequently regulates EMT of breast cancer cells [25,26]. Additionally, the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway, which is implicated in various tumors, is associated with tumor metastasis and proliferation [27,28]. PIM2 is involved in carcinogenesis, and plays a role in modulating informatory capacity and aerobic glycolysis through the PIM2/mTORC1 signaling pathway [29,30,31]. The present study, in conjunction with previous findings, supported the hypothesis that *H. pylori* infection and its CagA may enhance PIM2 expression in gastric cells via the JAK/STAT signaling pathway. This interaction disrupts the balance between cellular proliferation and apoptosis, and upregulate EMT in gastric epithelial cells by the mTORC1 signaling pathway, thereby predisposing the stomach be predisposed to carcinogenesis.

Additionally, the present study demonstrated that co-culture of *H. pylori* and GC cells resulted in the overexpression of PIM2 in the infected GC cells. This effect was observed not only with the *H. pylori* NCTC11637 (*cagA*+) strain but also with the *H. pylori* mutant strain NCTC11637 (Δ*cagA*) , albeit to a lesser extent. These findings suggest that *H. pylori* may produce bioactive components other than CagA that are capable of regulating PIM2 expression. Identifying and characterizing these bacterial components could provide new insights into the unknown pathogenic mechanisms of *H. pylori* infection, and should be a focus of future research.

The present study has limitations. Firstly, the current study was confined to the assessment of PIM2 mRNA expression within gastric cancer cell lines, which may not accurately represent the conditions in the gastric mucosa. The findings, therefore, should be interpreted with caution. Secondly, the study has focused exclusively on the alterations in PIM2 mRNA levels, not accounting for potential changes at the protein level, which may lead to an incomplete understanding of the effects of *cagA* transfection on the expression and functionality of PIM2 in gastric cancer cells.

5. Conclusions

Our study unveiled that the expression of PIM2 in gastric cancer cells was influenced by both the quantity and duration of *H. pylori* infection. Furthermore, we pinpointed that *cagA* of *H. pylori* played a pivotal role in upregulating PIM2 expression in gastric cancer epithelial cells. Our results further illustrated that transfection of *cagA* into gastric cancer cells elevated PIM2 expression, providing additional evidence for the regulatory impact of *cagA* on PIM2 expression in gastric cancer epithelial cells. Considering the oncogenic nature of PIM2, our findings imply that *cagA*-mediated regulation of PIM2 expression may constitute an important mechanism underlying the pathogenesis of *H. pylori* infection-induced gastric cancer.

Author contributions

Conception: HW and RZ.
Interpretation or analysis of data: WL.
Preparation of the manuscript: HW, SC.
Revision for important intellectual content: WL, WC, LH.
Supervision: WL and RZ.

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Informed consent statement

Not applicable.

Conflict of interest

No conflict of interest.

Supplementary data

The supplementary files are available to download from http://dx.doi.org/10.3233/CBM-230351.

References

[1] Y. Song, X. Liu, W. Cheng, H. Li and D. Zhang, The global, regional and national burden of stomach cancer and its attributable risk factors from 1990 to 2019, *Scientific Reports* **1**(12) (2022), 11542.

[2] X. Guo, B. Yin, C. Wang, H. Huo and Z. Aziziaram, Risk assessment of gastric cancer in the presence of Helicobacter pylori cagA and hopQII genes, *Cellular and Molecular Biology* **4**(67) (2022), 299–305.

[3] N. Ito, H. Tsujimoto, H. Ueno, Q. Xie and N. Shinomiya, Helicobacter pylori-mediated immunity and signaling transduction in gastric cancer, *Journal of Clinical Medicine* **11**(9) (2020), 3699.

[4] Y. Wen, H. Huang, T. Tang, H. Yang, X. Wang, X. Huang et al., AI-2 represses CagA expression and bacterial adhesion, attenuating the Helicobacter pylori-induced inflammatory response of gastric epithelial cells, *Helicobacter* **2**(26) (2021), e12778.

[5] A. Lamb, X.D. Yang, Y.H. Tsang, J.D. Li, H. Higashi, M. Hatakeyama et al., Helicobacter pylori CagA activates NF-kappaB by targeting TAK1 for TRAF6-mediated Lys 63 ubiquitination, *EMBO Reports* **10**(11) (2009), 1242–1249.

[6] F. Wang, N. Qu, J. Peng, C. Yue, L. Yuan and Y. Yuan, CagA promotes proliferation and inhibits apoptosis of GES-1 cells by upregulating TRAF1/4-1BB, *Molecular Medicine Reports* **2**(16) (2017), 1262–1268.

[7] S. Chen, G. Duan, R. Zhang and Q. Fan, Helicobacter pylori cytotoxin-associated gene A protein upregulates alpha-enolase expression via Src/MEK/ERK pathway: implication for progression of gastric cancer, *International Journal of Oncology* **2**(45) (2014), 764–770.

[8] N.K, Panchal and E.P Sabina, A serine/threonine protein PIM kinase as a biomarker of cancer and a target for anti-tumor therapy, *Life Sciences* **15**(255) (2020), 117866.

[9] X. Zhang, M. Song, J.K. Kundu, M.H. Lee and Z.Z. Liu, PIM kinase as an executional target in cancer, *Journal of Cancer Prevention* **3**(23) (2018), 109–116.

[10] V. Asati, D.K. Mahapatra and S.K. Bharti, PIM kinase inhibitors: Structural and pharmacological perspectives, *European Journal of Medicinal Chemistry* **6**(172) (2019), 95–108.

[11] J.C. Wang, D.P. Chen, S.X. Lu, J.B. Chen, Y. Wei, X.C. Liu et al., PIM2 expression induced by proinflammatory macrophages suppresses immunotherapy efficacy in hepatocellular carcinoma, *Cancer Research* **18**(82) (2022), 3307–3320.

[12] Y. Wang, J. Xiu, C. Ren and Z. Yu, Protein kinase PIM2: A simple PIM family kinase with complex functions in cancer metabolism and therapeutics, *Journal of Cancer* **9**(12) (2021), 2570–2581.

[13] C. Lu, P. Qiao, Y. Sun, C. Ren and Z. Yu, Positive regulation of PFKFB3 by PIM2 promotes glycolysis and paclitaxel resistance in breast cancer, *Clinical and Translational Medicine* **4**(11) (2021), e400.

[14] X. Li, K. Pan, M. Vieth, M. Gerhard, W. Li and R. Mejias-Luque, JAK-STAT1 signaling pathway is an early response to helicobacter pylori infection and contributes to immune escape and gastric carcinogenesis, *International Journal of Molecular Sciences* **8**(23) (2022), 4147.

[15] P.S. Mary, W. Chaiwangyen, M. Weber, B. Al-Kawlani, R.R. Favaro, U. Jeschke et al., PIM kinases 1, 2 and 3 in intracellular LIF signaling, proliferation and apoptosis in trophoblastic cells, *Experimental Cell Research* **1**(359) (2017), 275–283.

[16] N. Murata-Kamiya and M. Hatakeyama, Helicobacter pylori-induced DNA double-stranded break in the development of gastric cancer, *Cancer Science* **6**(113) (2022), 1909–1918.

[17] S.Y. Chen, R.G. Zhang and G.C. Duan, Pathogenic mechanisms of the oncoprotein CagA in H. pylori-induced gastric cancer, *Oncology Reports* **6**(36) (2016), 3087–3094.

[18] Z. Ge, Y.L. Zhu, X. Zhong, J.K. Yu and S. Zheng, Discovering differential protein expression caused by CagA-induced ERK pathway activation in AGS cells using the SELDI-ProteinChip platform, *World Journal of Gastroenterology* **4**(14) (2008), 554–562.

[19] R. Lu, Y.G. Zhang and J. Sun, STAT3 activation in infection and infection-associated cancer, *Molecular and Cellular Endocrinology* **8**(451) (2017), 80–87.

[20] I.O. Lee, J.H. Kim, Y.J. Choi, M.H. Pillinger, S.Y. Kim, M.J. Blaser et al., Helicobacter pylori CagA phosphorylation status determines the gp130-activated SHP2/ERK and JAK/STAT signal transduction pathways in gastric epithelial cells, *Journal of Biological Chemistry* **21**(285) (2010), 16042–50.

[21] A. Sukri, A. Hanafiah, N.R. Kosai, T.M. Mohammed and R. Mohamed, New insight on the role of Helicobacter pylori cagA in the expression of cell surface antigens with important biological functions in gastric carcinogenesis, *Helicobacter* **5**(27) (2022), e12913.

[22] I.S. Sougleri, K.S. Papadakos, M.P. Zadik, M. Mavri-Vavagianni, A.F. Mentis and D.N. Sgouras, Helicobacter pylori CagA protein induces factors involved in the epithelial to mesenchymal transition (EMT) in infected gastric epithelial cells in an EPIYA- phosphorylation-dependent manner, *Febs Journal* **2**(283) (2016), 206–220.

[23] N.M. Santio and P.J. Koskinen, PIM kinases: From survival factors to regulators of cell motility, *International Journal of Biochemistry and Cell Biology* **12**(93) (2017), 74–85.

[24] C.L. Chaffer, J.B. San, E. Lim and R.A. Weinberg, EMT, cell plasticity and metastasis, *Cancer and Metastasis Reviews* **4**(35) (2016), 645–654.

[25] N. Uddin, R.K. Kim, K.C. Yoo, Y.H. Kim, Y.H. Cui, I.G. Kim et al., Persistent activation of STAT3 by PIM2-driven positive feedback loop for epithelial-mesenchymal transition in breast cancer, *Cancer Science* **6**(106) (2015), 718–725.

[26] M. Rokavec, M.G. Oner, H. Li, R. Jackstadt, L. Jiang, D. Lodygin et al., IL-6R/STAT3/miR-34a feedback loop promotes EMT-mediated colorectal cancer invasion and metastasis, *Journal of Clinical Investigation* **4**(124) (2014),1853–1867.

[27] C. Cloninger, A. Bernath, T. Bashir, B. Holmes, N. Artinian, T. Ruegg et al., Inhibition of SAPK2/p38 enhances sensitivity to mTORC1 inhibition by blocking IRES-mediated translation initiation in glioblastoma, *Molecular Cancer Therapeutics* **12**

(10) (2011), 2244–2256.
[28] R.A. Cairns, I.S. Harris and T.W. Mak, Regulation of cancer cell metabolism, *Nature Reviews Cancer* **2**(11) (2011), 85–95.
[29] G. Yin, Y. Li, M. Yang, X.M. Cen and Q.B. Xie, Pim-2/mTORC1 Pathway Shapes Inflammatory Capacity in Rheumatoid Arthritis Synovial Cells Exposed to Lipid Peroxidations, *Biomed Research International* **3**(2015) (2015), 240210.
[30] M. Liao, F. Hu, Z. Qiu, J. Li, C. Huang, Y. Xu and X. Cheng, Pim-2 kinase inhibits inflammation by suppressing the mTORC1 pathway in atherosclerosis, *Aging* **18**(13) (2021), 22412–22431.
[31] X.H. Zhang, H.L. Yu, F.J. Wang, Y.L. Han and W.L. Yang, Pim-2 Modulates Aerobic Glycolysis and Energy Production during the Development of Colorectal Tumors, *International Journal of Medical Sciences* **6**(12) (2015), 487–93.