Short Communication

The Evaluation of Gene Expression of *GPR83*, *CA1*, *AWP1*, and *WTAP* in AGS Cells Stimulated by the *Helicobacter pylori cagC* Gene

Abbas Doosti *, Zahra Khoshfetrat
Department of Biology, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

Received 13 October 2022 Accepted 15 April 2023

Abstract

Gastric carcinoma (GC) is the third leading cause of malignancy-related deaths worldwide, and *Helicobacter pylori* is one of the identified causes of gastric cancer. The Cag pathogenicity island (*cagA*, *cagC*, *virB2*) is one of the major pathogens of *H. pylori*, which increased the risk of gastric cancer development. Some studies have shown that *H. pylori* significantly alters the expression of certain genes in gastric epithelial cells. This study aimed to investigate the expression of the *GPR83*, *CA1*, *AWP1*, and *WTAP* genes in AGS cells transfected with the recombinant pIRES2-EGFP-cagC vector. The pIRES2-EGFP-cagC and pIRES2-EGFP plasmids (as controls) were transfected into AGS cells by lipofectamine 2000 solution. Then, RNA extraction and cDNA synthesis were performed. The expression of *GPR83*, *CA1*, *AWP1*, and *WTAP* genes was evaluated using the real-time PCR method. Finally, the expression of each gene was evaluated using SPSS software and t-test independent statistical tests. Our findings indicated that the expression of the *GPR83* gene in AGS cells treated with *cagC* statistically significantly increased compared to control cells (P=0.0327). On the contrary, the *WTAP* expression was significantly decreased (P=0.0132), whereas the AWP1 and CA1 mRNA expression levels were not statistically significant. This study shows that *GPR83* and *WTAP* genes's expression in the host is significantly altered through *cagC* gene expression. Hence, it seems that the *cagC* gene's presence can explain some alterations in the expression of gastric epithelial cell genes and the cause of gastric cancer pathogenesis caused by *H. pylori* infection.

***Keywords*:** *cagC* gene, Gastric carcinoma, *GPR83*, *Helicobacter pylori*, *WTAP*

Introduction

Gastric cancer (GC) is one of the most critical public health issues, ranking fifth among the most common cancers and is considered the third leading cause of death due to cancer(Assumpção et al., 2020). Unfortunately, the symptoms of this disease appear in advanced stages, and the survival rate varies relatively between countries, which is probably increased by timely diagnosis and early tumor resection (Johnston and Beckman, 2019). GC is the result of a multifactorial process involving numerous environmental (*Helicobacter pylori* infection, alcohol consumption, tobacco abuse, advanced age, and poor diets) and genetic factors, and its global decline requires a healthy diet, early screening and, of course, the elimination of *H. pylori* (Eusebi et al., 2020; Massarrat and Stolte, 2014). *Helicobacter pylori* is a microaerophilic, spiral, and gram-negative bacterium that usually reported in the human stomach and induces gastroduodenal ulcers, gastritis, intestinal metaplasia, gastric atrophy, and gastric cancer(Alfarouk et al., 2019; Malfertheiner et al., 2017). The pathogenicity of *H. pylori* could be increased by cag pathogenicity island (*cag-PAI*) genes (Amieva and Peek Jr, 2016). Interestingly this pathogenicity island is not usually found in *H. pylori* strains isolated from asymptomatic humans(De Witte et al., 2022). *H. pylori* obtained cag-PAI through horizontal transfer from other bacterial species and encodesthe secretory system type IV (TSS4). CagA oncoprotein enters the gastric epithelial cells via TSS4. It interacts with several cellular proteins, leading to dysfunction of actin and cytoskeleton intracellular signaling, adherence to adjacent cells, increased cell motility(Churin et al., 2003; De Witte et al., 2022), phenotypic alteration, and finally gastritis GC(Backert and Selbach, 2008; Lin et al., 2020). The CagC protein is one of the TSS4 components that localized to the surface *of H. pylori* and plays an essential role in the extracellular pilus formation. The pilus interacts with the integrin receptor on gastric epithelial cells and facilitates the injection of *H. pylori* virulence factors into the gastric epithelial cell(Chang et al., 2018). Many studies have indicated that gene expression of some

*Corresponding author's e-mail address: abbas.doosti47@yahoo.com

gastric epithelial cells is significantly altered after *H. pylori* adhesion, which might explain the cause of gastritis due to *H. pylori* infection(Heidari and Doosti, 2020). This study aimed to investigate the expression of the *GPR83* (G protein-coupled receptor 83), *CA1* (carbonic anhydrase 1), *AWP1* (associated with PRK1), and *WTAP* (Wilms tumor1-associating protein) genes in AGS cells transfected with the recombinant pIRES2-EGFP-cagC vector.

Materials and Methods

pIRES2-EGFP-cagC plasmid propagation & purification

Recombinant plasmids pIRES2-EGFP-cagC and pIRES2-EGFP were prepared at the Biotechnology Research Center of the Islamic Azad University of Shahrekord. Plasmid amplification was performed by transferring to a bacterial host, *Escherichia coli* (TOP10). The pIRES2-EGFP-*cagC* vector was purified and visualized by 1% agarose gel electrophoresis. The PCR technique was applied to confirm the plasmid's accuracy after being extracted from the bacterium, and double digestion by restriction enzymes SalI /SacII was used to confirm the cagC gene's presence in the plasmid pIRES2-EGFP.

AGC cell line expansion

A human gastric epithelial cell line, AGS (gastric adenocarcinoma) was obtained from the Pasteur Institute of Iran and was grown under standard cell culture conditions (5% CO2, 37°C) in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) and penicillin-streptomycin 1%.

Transfection of pIRES2-EGFP-cagC into AGS cells

The lipofectamine 2000 (Thermo Fisher, USA) was used to transfect the cell lines based on the manufacturer's guidance. Briefly, 2 µl of Lipofectamine 2000 and 2 µg of plasmid were diluted in 100 µl of RPMI-reduced serum medium and incubated for 10 min at room temperature to allow the DNA-Lipofectamine2000 complexes to form. The complexes were added to cells, and after 4 h the medium was replaced with RPMI-1640 supplemented with 10% FBS. After 24 h, 1% Pen/Strep and 600 µg/ml neomycin were added.

RNA preparation and real-time RT-PCR

In the present study, the RT-PCR technique was used to evaluate the altered expression of *GPR83*, *CA1*, *AWP1*, and *WTAP* genes in AGS cells transfected with pIRES2-EGFP-cagC vector. Total RNA was extracted from the cells and reverse-transcribed into cDNA. PCR method was used to confirm the extracted plasmid's accuracy and evaluate the cloned gene (cagC), using specific primers (Table 1). PCR cycling was performed in SYBR Green PCR Master Mix (Yekta Tajhiz, Iran). The expression of GAPDH was used to normalize that of the target genes.

Statistical analysis

Using SPSS version 22 software, the expression levels of *AWP1*, *CA1*, *WTAP*, and *GPR83* genes were analyzed. Statistical t-test was performed to compare the expression of the mentioned genes in samples compared to control cells at a significance level of 95% (P Value=0.05). P-value ≤0.05 was considered statistically significant.

Results

Plasmid confirmation by PCR and enzymatic digestion

The *cagC* gene-specific primers amplified PCR products with 360 bp size. Double digestion was used to confirm the *cagC* gene's presence in the plasmid pIRES2-EGFP by restriction enzymes SalI / SacII, and in the following, the 5308 bp fragment vector and a 360 bp *cagC* gene fragment were observed (Figure 1).

Table 1. Specific primer sequence to confirm the extracted plasmid's accuracy and evaluate the cloned gene (cagC)

Primer	sequence	Size (bp)
Forward	5'- TTAGGGCTAGTGATGACAACCAATC -3'	
Reverse	5'- CCTAAAGCACAACCACCTACGATC -3'	360

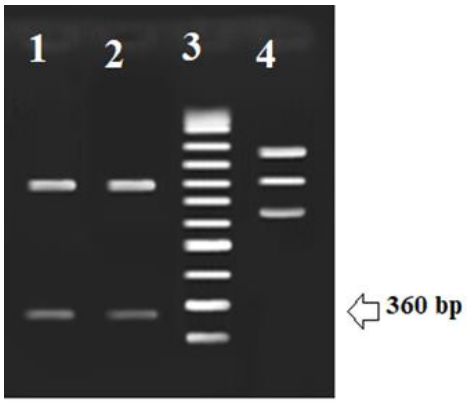

Figure 1. Enzymatic digestion of the vector containing *cagC* gene. line 3: The 100-bp marker of the Firentaz Company Formation, lines 1,2: The heavy fragment of 5308 bp corresponds to the vector, and the fragment of 360 bp corresponds to the cagC gene.

Discussion

Gastric carcinoma is a malignant disease with a generally poor prognosis. The majority of GCs are sporadic subtypes that are strongly associated with environmental risk factors. *Helicobacter pylori* infection is identified as the primary etiologic factor in gastrointestinal problems; it persistently colonizes gastric mucosa in about 50 percent of the world's adult population, but most persons remain asymptomatic(Chang et al., 2018). *H. pylori's* virulence strain contains a 40-kb chromosomal region known as the Cag pathogenicity island (PAI)(Cover et al., 2020). It encodes about 31 genes that form a type IV secretion system and a needle-like structure (pilus) for connecting to the cytoplasm of the epithelial gastric cell and injection of virulence factors(Craig et al., 2019). Approximately 95% of *H. pylori* isolated from East Asia were cag PAI-positive. The *cagC* is one of the *cagPAI* genes required for T4SS function, and T4SS causes numerous alterations in gastric epithelial cells(Fischer et al., 2020) . The cagC gene interact with integrin α5β1 receptor on gastric epithelial cells via CagL, guiding proper positioning of the T4SS and facilitating translocation of cagA to the cytoplasm of the gastric epithelial cell(Chang et al., 2018). Lim et al. used the AGS cell line infected with *H. pylori* to monitor gene expression changes associated with *H. pylori* adhesion to gastric cancer cells. They showed that *H. pylori* altered the expression of genes involved in metabolism, transcription factors, and structural proteins, as well as known growth factor receptors, oncogenes, and tumor suppressor genes(Lim et al., 2003). Guillemin et al. have shown that the cag T4SS has an essential role in proinflammatory activation of gastric epithelial cells and results in upregulated expression of multiple cytokines, including the proinflammatory cytokine interleukin 8 (IL-8)(Guillemin et al., 2002). Identification of the pathogen-host interactions at the gene level could improve our understanding of host and organism roles during pathogenesis. Hence, this study analyzed the expression of *GPR83*, *CA1*, *AWP1*, and *WTAP* genes in AGS cells transfected with the recombinant pIRES2-EGFP-cagC vector. Our results showed that the cagC gene's presence could lead to significant expression changes in *GPR83* and *WTAP* genes of AGS cells. The *WTAP* gene encodes the Wilms tumor 1-associated protein, which has a ubiquitous expression pattern. WTAP and WT1 are distributed in the nucleoplasm and nuclear speckles, where they are partially co-localized with splicing factors(Fan et al., 2022), regulating both the cell cycle and alternative splicing by making a protein complex Based on the downregulated expression of the *WTAP* gene in AGS cells, we suggested that reduced WTAP likely leads to alteration of RNA processing and post-transcriptional regulation of multiple cellular proteins, thus might be involved in the pathophysiology of GC. GPR83 is a member of the G-protein coupled-receptors superfamily, which is responsible for the initiation of intracellular signaling cascades(Lueptow et al., 2018). Increased expression of the *GPR83* gene probably leads to increased distribution of *GPR83* on the surface of gastric epithelial cells. It likely can lead to the out-of-control launch of its related intracellular signaling cascades and disruption of cell physiological balance. Overall, these results suggest that *GPR83* and *WTAP* could be considered new therapeutic targets for *H. pylori*-induced gastric malignancy and open new insight into cancer treatment.

Conclusions

These results suggest that *H. pylori* adhesion to AGS cells leads to the altered expressions of multiple cellular proteins, which trigger the inflammatory reactions. The study of these changes in the pathogen's presence can provide an accurate description of the pathogenesis and new therapies based on these genetic changes.

Conflict of Interests

No conflict or competing financial interests exist.

References

Alfarouk K. O., Bashir A. H., Aljarbou A. N., Ramadan A. M., Muddathir A. K., AlHoufie S. T.,et al. (2019) The Possible Role of Helicobacter pylori in Gastric Cancer and Its Management. Frontiers in oncology 9:75.

Amieva M. and Peek Jr R. M. (2016) Pathobiology of Helicobacter pylori-Induced Gastric Cancer. Gastroenterology 150:64-78.

Assumpção P. P., Barra W. F., Ishak G., Coelho L. G. V., Coimbra F. J. F., Freitas H. C., et al. (2020) The diffuse-type gastric cancer epidemiology enigma. BMC gastroenterology 20:223.

Backert S. and Selbach M. (2008) Role of type IV secretion in Helicobacter pylori pathogenesis. Cellular microbiology 10:1573-1581.

Chang W.-L., Yeh Y.-C. and Sheu B.-S. (2018) The impacts of H. pylori virulence factors on the development of gastroduodenal diseases. Journal of biomedical science 25:68.

Churin Y., Al-Ghoul L., Kepp O., Meyer T. F., Birchmeier W. and Naumann M. (2003) Helicobacter pylori CagA protein targets the c-Met receptor and enhances the motogenic response. The Journal of cell biology 161:249-255.

Cover T. L., Lacy D. B. and Ohi M. D. (2020) The Helicobacter pylori Cag Type IV Secretion System. Trends in microbiology 28:682-695.

Craig L., Forest K. T. and Maier B. (2019) Type IV pili: dynamics, biophysics and functional consequences. Nature reviews. Microbiology 17:429-440.

De Witte C., Berlamont H. and Haesebrouck F. (2022) Helicobacter. Pathogenesis of Bacterial Infections in Animals:413-432.

Eusebi L. H., Telese A., Marasco G., Bazzoli F. and Zagari R. M. (2020) Gastric cancer prevention strategies: A global perspective. Journal of gastroenterology and hepatology 35:1495-1502.

Fan Y., Li X., Sun H., Gao Z., Zhu Z. and Yuan K. (2022) Role of WTAP in Cancer: From Mechanisms to the Therapeutic Potential. Biomolecules 12:1224.

Fischer W., Tegtmeyer N., Stingl K. and Backert S. (2020) Four Chromosomal Type IV Secretion Systems in Helicobacter pylori: Composition, Structure and Function. Frontiers in microbiology 11:1592.

Guillemin K., Salama N. R., Tompkins L. S. and Falkow S. (2002) Cag pathogenicity island-specific responses of gastric epithelial cells to Helicobacter pylori infection. Proceedings of the National Academy of Sciences of the United States of America 99:15136-15141.

Heidari F. and Doosti A. (2020) Evaluation of GPR83 Gene Expression in AGS Cells Transfected with Pflag-CMV-3-TagD Recombinant Vector. New Cellular and Molecular Biotechnology Journal 10:39-48.

Johnston F. M. and Beckman M. (2019) Updates on Management of Gastric Cancer. Current oncology reports 21:67.

Lim J. W., Kim H. and Kim K. H. (2003) Cell adhesion-related gene expression by Helicobacter pylori in gastric epithelial AGS cells. The international journal of biochemistry & cell biology 35:1284-1296.

Lin A. S., Dooyema S. D., Frick-Cheng A. E., Harvey M. L., Suarez G., Loh J. T., et al. (2020) Bacterial Energetic Requirements for Helicobacter pylori Cag Type IV Secretion System-Dependent Alterations in Gastric Epithelial Cells. Infection and immunity 88:e00790-00719.

Lueptow L. M., Devi L. A. and Fakira A. K. (2018) Targeting the Recently Deorphanized Receptor GPR83 for the Treatment of Immunological, Neuroendocrine and Neuropsychiatric Disorders. Progress in molecular biology and translational science 159:1-25.

Malfertheiner P., Megraud F., O'Morain C., Gisbert J., Kuipers E., Axon A., et al. (2017) Management of Helicobacter pylori infection-

the Maastricht V/Florence Consensus Report. Gut 66:6-30.

Massarrat S. and Stolte M. (2014) Development of gastric cancer and its prevention. Archives of Iranian medicine 17:514-520.