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

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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; Malferttheiner 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 (Buckert 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
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>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Size (bp)</th>
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<tbody>
<tr>
<td>Forward</td>
<td>5’-TTAGGGCTAGTGTGACAACCCAATC-3’</td>
<td></td>
</tr>
<tr>
<td>Reverse</td>
<td>5’-CCTAAAGCACAACCACCTACGATC-3’</td>
<td>360</td>
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Table 1. Specific primer sequence to confirm the extracted plasmid's accuracy and evaluate the cloned gene (cagC)
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et al. have shown that the cag T4SS has an essential tumor suppressor genes as known growth factor receptors, oncogenes, and transcription factors, and structural proteins, as well expression of genes involved in metab cells. They showed that associated with H. pylori 2018) cytoplasm of the gastric epithelial cell and facilitating translocation of cagA to the via CagL, guiding proper positioning of the T4SS with integrin α5β1 receptor on gastric epithelia numerous alterations in gastric epithelial required for T4SS function, and T4SS causes PAI 95% of virulence factors of the epithelial gastric cell and injection of like structure (pilus) for connecting to the cytoplasm that form a type IV secretion system and a needle (PAI) region known as the Cag pathogenicity island virulence strain contains a 40 kb chromosomal altered the G. 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, AWPI, and WTAP genes in AGS cells transfected with the recombinant pIRE2-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.

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

Conflict of Interests

No conflict or competing financial interests exist.

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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.
References


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