Helicobacter pylori tumor necrosis factor-α inducing protein promotes cytokine expression via nuclear factor-κB

Chun-Li Tang, Bo Hao, Guo-Xin Zhang, Rui-Hua Shi, Wen-Fang Cheng

AIM: To study the effects of Helicobacter pylori (H. pylori) tumor necrosis factor-α (TNF-α) inducing protein (Tip-α) on cytokine expression and its mechanism.

METHODS: We cloned Tip-α from the H. pylori strain 26695, transformed Escherichia coli with an expression plasmid, and then confirmed the expression product by Western blotting. Using different concentrations of Tip-α that affected SGC7901 and GES-1 cells at different times, we assessed cytokine levels using enzyme-linked immunosorbent assay. We blocked SGC7901 cells with Tip-α interference, whether recombinant Tip-α protein was recombined successfully. The levels of IL-1β, IL-8 and TNF-α levels were significantly decreased compared to cells that only underwent Tip-α interference (P < 0.05).

RESULTS: Western blot analysis using an anti-Tip-α antibody revealed a 23-kDa protein, which indicated that recombinant Tip-α protein was recombined successfully. The levels of IL-1β, IL-8 and TNF-α were significantly higher following Tip-α interference, whether GES-1 cells or SGC-7901 cells were used (P < 0.05).

However, the levels of cytokines (including IL-1β, IL-8 and TNF-α) secreted by SGC-7901 cells were greater than those secreted by GES-1 cells following treatment with Tip-α at the same concentration and for the same duration (P < 0.05). After blocking NF-κB with PDTC, the levels of cytokines (GES-1 cells and SGC-7901 cells) underwent interference with Tip-α. We found that IL-1β and TNF-α levels were significantly decreased compared to cells that only underwent Tip-α interference (P < 0.05).

CONCLUSION: Tip-α plays an important role in cytokine expression through NF-κB.

© 2013 Baishideng. All rights reserved.

Key words: Helicobacter pylori; Tumor necrosis factor-α inducing protein; Interleukin-1β; Interleukin-8; Tumor necrosis factor-κB

INTRODUCTION

Infection with Helicobacter pylori (H. pylori) leads to chronic gastritis, peptic ulcer, and gastric lymphoma[1-3]. H. pylori has also been associated with gastric cancer[4], and H. pylori exerts its pathogenesis by secreting toxins, including hemolysin, lipopolysaccharides, CagA and VacA[5-9]. CagA and VacA are major virulence factors. Persistent infection by H. pylori enables these toxins to stimulate gastric epithelial cells to produce a large number of cytokines such as tumor necrosis factor (TNF-α) and interleukin 1, 6 and 8 (IL-1, IL-6 and
IL-8, thus generating an inflammatory reaction\(^{10-14}\). Tumor necrosis factor-\(\alpha\) inducing protein (Tip-\(\alpha\)) is a new toxin discovered recently, and likely accelerates the inflammation and cancers caused by \(H.\ pylori\)\(^{13}\). However, its function and the mechanism underlying these effects remain unclear. The present work was conducted to determine the effects of recombinant Tip-\(\alpha\) (rTip-\(\alpha\)) on human gastric epithelial cells and gastric cancer cytokine expression, as well as explore the mechanisms involved.

**MATERIALS AND METHODS**

**Materials**

\(H.\ pylori\) strain 26695 was obtained from the Shanghai Institute of Digestive Disease. The following reagents were used in this study: Dual Promoter TA Cloning Kit pCR 4 II and pET28a vectors (Invitrogen); monoclonal rabbit anti-Tip-\(\alpha\) antibody (Beijing Aviva Systems Biology); BamHI I, Xho I and Prestained Protein Molecular Weight Markers (Fermentas); DNA and gel extraction kit from Tiangen Biotech (Beijing) Co. Ltd.; DNA marker (TaKaRa); His TrapTM H. pylori affinity chromatography column (GE Healthcare); and enhanced chemiluminescence kit (Pierce Protein Biology Products). The polymerase chain reaction primer sequences were 5’-TTGGATCCATGGCTGCAGGCTTG-3’, which contained an Xho I restriction site, and 5’-GGCTCGAGCATGCTGATAG-3’, which contained a BamHI I restriction site. The primers were synthesized by Invitrogen. The human gastric epithelial cell line GES-1 and gastric cancer SGC7901 cells were purchased from the Shanghai Cancer Institute. Enzyme-linked immunosorbent assay (ELISA) kits were obtained from MultiSciences Biotech (Shanghai) Co., Ltd., while pyrrolidine dithiocarbamate (PDTC) was purchased from MultiSciences Biotech (Shanghai) Co., Ltd.}

**Methods**

**Expression, purification, and identification of Tip-\(\alpha\):**

We cloned Tip-\(\alpha\) from the genome of \(H.\ pylori\) strain 26695. The Tip-\(\alpha\) gene and pET28a vector (His tag) were digested with BamHI I and Xho I, purified, and then ligated together to generate the ET28a-Tip-\(\alpha\) plasmid expressing recombinant Tip-\(\alpha\). This plasmid was transformed into \(Escherichia\ coli\) and the resultant protein was purified by Ni-NTA affinity chromatography and verified by Western blotting.

**Cell recovery, culture, and passage:** Cryopreserved GES-1 and SGC-7901 cells were centrifuged at 1000 rpm for 5 min. After removal of the supernatant, these cells were cultured in 60 mm \(\times\) 60 mm dishes containing Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum.

**IL-1\(\beta\), IL-8 and TNF-\(\alpha\) levels at different times following interference by \(12.5\ \mu g/mL\) rTip-\(\alpha\) in GES-1 and SGC7901 cells:**

GES-1 and SGC7901 cells during their logarithmic growth phase underwent interference with \(12.5\ \mu g/mL\) rTip-\(\alpha\) after starvation in serum-free medium for 24 h. The levels of IL-1\(\beta\), IL-8 and TNF-\(\alpha\) cytokines were then assessed at 0, 1, 2, 4 and 8 h post-interference using ELISA.

**Levels of IL-1\(\beta\), IL-8 and TNF-\(\alpha\) in GES-1 and SGC7901 cells following incubation with different concentrations of rTip-\(\alpha\):**

We incubated GES-1 and SGC7901 cells with the following concentrations of rTip-\(\alpha\): 0, 12.5, 25 and 50 \(\mu g/mL\). After 2 h, we examined the levels of IL-1\(\beta\), IL-8 and TNF-\(\alpha\) using ELISA.

**Effects of rTip-\(\alpha\) on IL-1\(\beta\) and TNF-\(\alpha\) expression after PDTC-mediated inhibition of NF-\(\kappa\)B:**

Four groups consisting of the same number of GES-1 and SGC7901 cells were starved in serum-free medium for 24 h before undergoing different treatments. Group A was treated with 12.5 \(\mu g/mL\) rTip-\(\alpha\) for 2 h. Group B was treated similarly after PDTC blocking of NF-\(\kappa\)B for 4 h. Groups C and D were incubated with serum-free medium and dimethyl sulfoxide (the vehicle with which PDTC was diluted), respectively. ELISA was performed to detect the levels of IL-1\(\beta\) and TNF-\(\alpha\) in each group.

**Statistical analysis**

Data are presented as the mean ± SD and analyzed using SPSS 17.0. The Student’s \(t\) test was used to compare two groups, while one-way analysis of variance was used to compare among several groups. A \(P\) value < 0.05 was considered statistically significant.

**RESULTS**

**Identification by Western blotting after rTip-\(\alpha\) expression and purification**

Western blotting analysis demonstrated that the Tip-\(\alpha\) recombinant protein and anti-human Tip-\(\alpha\) monoclonal antibody could be specifically bound; specific bands were found (Figure 1). Western blotting analysis by non-denaturing gel electrophoresis showed active dimer bands (46 kDa).
IL-1β, IL-8 and TNF-α levels at different times following interference with different concentrations of recombinant tumor necrosis factor-α inducing protein in GES-1 and SGC7901 cells

The levels of IL-1β, IL-8 and TNF-α were significantly higher after GES-1 and SGC7901 cells underwent interference with 12.5 μg/mL rTip-α for 1, 2, 4 and 8 h than those at 0 h. Cytokine secretion by GES-1 and SGC7901 cells peaked after rTip-α interference for 2 h, the levels of IL-8 (2.53 ± 0.50) and TNF-α (1.41 ± 0.10) in SGC7901 cells were significantly higher than those in GES-1 cells (0.84 ± 0.11 for IL-8 and 0.72 ± 0.08 for TNF-α). As shown in Table 1, the levels of IL-1β in GES-1 and SGC7901 cells (2.07 ± 0.10 and 2.07 ± 0.30, respectively) were not statistically different after rTip-α interference for 2 h.

Levels of IL-1β, IL-8 and TNF-α in GES-1 and SGC7901 cells following incubation with different concentrations of rTip-α

The levels of IL-1β, IL-8 and TNF-α were significantly higher than those in the blank control in GES-1 and SGC7901 cells after rTip-α interference for 2 h. Cytokine secretion of GES-1 and SGC7901 cells peaked at 12.5 μg/mL, suggesting that this effect was not concentration-dependent (Table 2).

Effects of rTip-α on IL-1β and TNF-α expression after PDTC-mediated inhibition of NF-κB

The levels of IL-1β and TNF-α in SGC7901 cells in Group B (treated with PDTC + rTip-α) were higher than those in Groups C and D (no rTip-α and PDTC), but markedly lower than those in Group A (only treated with rTip-α). As shown in Table 3, these differences were statistically significant (P < 0.05, F = 40.15).

Table 1  Cytokine levels at different times following interference of GES-1 and SGC7901 cells with 12.5 μg/mL recombinant tumor necrosis factor-α inducing protein

| Groups          | 0 h     | 1 h     | 2 h     | 4 h     | 8 h     |
|-----------------|---------|---------|---------|---------|---------|
| GES-1 (IL-1β)   | 0.34 + 0.04 | 0.88 + 0.09<sup>a</sup> | 2.07 + 0.30<sup>b</sup> | 1.35 + 0.20<sup>b</sup> | 1.41 + 0.15<sup>b</sup> |
| SGC-7901 (IL-1β)| 0.22 + 0.04 | 0.35 + 0.05 | 2.07 + 0.10<sup>b</sup> | 1.11 + 0.04<sup>b</sup> | 1.14 + 0.04<sup>b</sup> |
| GES-1 (IL-8)    | 0.35 + 0.05 | 0.60 + 0.12<sup>a</sup> | 0.84 + 0.11<sup>b</sup> | 0.64 + 0.06<sup>b</sup> | 0.50 + 0.07<sup>b</sup> |
| SGC-7901 (IL-8) | 0.70 + 0.02 | 0.78 + 0.19 | 2.53 + 0.50<sup>b</sup> | 2.26 + 0.24<sup>b</sup> | 2.14 + 0.68<sup>b</sup> |
| GES-1 (TNF-α)   | 0.39 + 0.06 | 0.39 + 0.07 | 0.72 + 0.08<sup>b</sup> | 0.53 + 0.03<sup>b</sup> | 0.51 + 0.14<sup>b</sup> |
| SGC-7901 (TNF-α)| 0.33 + 0.09 | 1.02 + 0.09<sup>b</sup> | 1.41 + 0.10<sup>b</sup> | 0.86 + 0.07<sup>b</sup> | 0.47 + 0.05<sup>b</sup> |

<sup>a</sup>P < 0.05 vs 0 h. IL-1β: Interleukin-1β; TNF-α: Tumor necrosis factor-α.

| Groups          | 2.5 μg/mL | 50 μg/mL |
|-----------------|-----------|----------|
| GES-1 (IL-1β)   | 0.59 + 0.11 | 2.07 + 0.39<sup>b</sup> | 2.26 + 0.09<sup>b</sup> | 1.23 + 0.13<sup>b</sup> |
| SGC-7901 (IL-1β)| 0.36 + 0.01 | 2.07 + 0.10<sup>b</sup> | 1.22 + 0.03<sup>b</sup> | 1.02 + 0.04<sup>b</sup> |
| GES-1 (IL-8)    | 0.39 + 0.08 | 0.84 + 0.11<sup>b</sup> | 0.75 + 0.09<sup>b</sup> | 0.61 + 0.15<sup>b</sup> |
| SGC-7901 (IL-8) | 0.78 + 0.09 | 2.53 + 0.50<sup>b</sup> | 1.50 + 0.16<sup>b</sup> | 1.41 + 0.14<sup>b</sup> |
| GES-1 (TNF-α)   | 0.30 + 0.06 | 0.72 + 0.08<sup>b</sup> | 0.54 + 0.13<sup>b</sup> | 0.63 + 0.10<sup>b</sup> |
| SGC-7901 (TNF-α)| 0.26 + 0.18 | 1.41 + 0.10<sup>b</sup> | 0.62 + 0.07<sup>b</sup> | 0.62 + 0.02<sup>b</sup> |

<sup>b</sup>P < 0.05 vs group 0. IL-1β: Interleukin-1β; TNF-α: Tumor necrosis factor-α.

Table 2  Cytokine levels in GES-1 and SGC7901 cells after interference with different concentrations of recombinant tumor necrosis factor-α inducing protein for 2 h

| Groups          | 0.5 μg/mL | 12.5 μg/mL | 25 μg/mL | 50 μg/mL |
|-----------------|-----------|------------|----------|----------|
| GES-1 (IL-1β)   | 0.59 + 0.11 | 2.07 + 0.39<sup>b</sup> | 2.26 + 0.09<sup>b</sup> | 1.23 + 0.13<sup>b</sup> |
| SGC-7901 (IL-1β)| 0.36 + 0.01 | 2.07 + 0.10<sup>b</sup> | 1.22 + 0.03<sup>b</sup> | 1.02 + 0.04<sup>b</sup> |
| GES-1 (IL-8)    | 0.39 + 0.08 | 0.84 + 0.11<sup>b</sup> | 0.75 + 0.09<sup>b</sup> | 0.61 + 0.15<sup>b</sup> |
| SGC-7901 (IL-8) | 0.78 + 0.09 | 2.53 + 0.50<sup>b</sup> | 1.50 + 0.16<sup>b</sup> | 1.41 + 0.14<sup>b</sup> |
| GES-1 (TNF-α)   | 0.30 + 0.06 | 0.72 + 0.08<sup>b</sup> | 0.54 + 0.13<sup>b</sup> | 0.63 + 0.10<sup>b</sup> |
| SGC-7901 (TNF-α)| 0.26 + 0.18 | 1.41 + 0.10<sup>b</sup> | 0.62 + 0.07<sup>b</sup> | 0.62 + 0.02<sup>b</sup> |

<sup>b</sup>P < 0.05 vs group A. IL-1β: Interleukin-1β; TNF-α: Tumor necrosis factor-α.

DISCUSSION

Tip-α is a novel gene that was discovered recently in *H. pylori*. Located in the *H. pylori* 0596 open reading frame of the *H. pylori* 26695 strain, Tip-α is also called *H. pylori* 0596 protein. Its open reading frame is 519 bp in length and constitutes 173 amino acids. Tip-α has a molecular weight of 19 kDa and can form active homodimers with a molecular weight of 38 kDa through disulfide bonding<sup>13</sup>. Recent studies have found that Tip-α is associated with the adsorption and colonization of *H. pylori* in gastric mucosa<sup>14</sup>. Some studies have shown that the structure of Tip-α is different from penicillin binding proteins. Tip-α is composed of three closely linked domains that interact with other proteins and nucleic acids. In particular, the homodimer formed by cysteine C25 and C27 is essential for Tip-α to serve its role in the gastric mucosal acidic environment<sup>17</sup>. Detected by gene chip technology, expression of the chemokines Cc 12 Cc17, Cc120, Cx11 and Cx15 was enhanced after Tip-α treatment in gastric cancer cells and gastric epithelial cells<sup>18</sup>. These chemokines can induce chemotaxis of immune cells to local sites of infection, resulting in immune regulation and pathology, and ultimately the inflammatory response<sup>19</sup>. Because our pET28a-Tip-α vector also expresses a 3.74 kDa His tag, the recombinant Tip-α protein we produced possesses a molecular weight of about 23 kDa and can form active homodimers with a molecular weight of 46 kDa through disulfide bonding. Our work indicates that after affecting gastric epithelial and cancer
cells, rTip-α can promote the expression of IL-1β, IL-8 and TNF-α. These cytokines are important in promoting the inflammatory response, thus linking Tip-α to inflammation and the occurrence of _H. pylori_-related gastrointestinal diseases. After incubating the cells for 2 h with rTip-α, the levels of cytokine expression peaked at 12.5 μg/mL, which was the best concentration for interference. The levels of cytokine expression induced by rTip-α were not time- or concentration-dependent. These results suggest that Tip-α affects the host by inducing toxin secretion into the exterior environment of the bacteria through the type II secretion system[21]. The toxins then enter host cells via receptor-mediated endocytosis instead of through injection via the IV secretion system[21-23]. Studies have shown that Tip-α possesses DNA binding activity. In particular, DNA affecting gastric mucosal cells can combine with some transcription factors to promote IL-1β, IL-8 and TNF-α expression, leading to the occurrence and development of _H. pylori_-related gastrointestinal diseases[24].

PDTC is a specific NF-κB inhibitor that works by blocking degradation of the NF-κB p65 subunit or IκB, thereby reducing NF-κB nuclear translocation[25]. Our data demonstrated no significant increase in IL-1β and TNF-α levels after pretreatment of SGC7901 cells with PDTC followed by rTip-α interference. This finding suggests that the promotion of cytokine expression by Tip-α may be regulated by NF-κB. However, further study is required to determine the underlying mechanism.

In addition, we found that when gastric epithelial and cancer cells were treated with the same concentration of Tip-α for the same duration, the level of cytokine expression in gastric cancer SGC7901 cells was significantly higher than that in gastric epithelial GE1-1 cells. This difference may be due to differential effects of rTip-α on NF-κB expression in the cell types or may be related to variations in the DNA binding activity of Tip-α in the cells. Some studies suggest that the increased cytokine expression promoted by Tip-α may hasten the invasion and metastasis of gastric cancer[28]. Overall, Tip-α can activate cytokine expression in an NF-κB-dependent manner. Tip-α plays a major role in the pathogenesis of _H. pylori_, however, its mechanism requires further investigation.

**COMMENTS**

**Background**

_Helicobacter pylori_ (_H. pylori_) exerts its pathogenesis by secreting toxins. Tumor necrosis factor-α (TNF-α) inducing protein (Tip-α) is a new toxin discovered recently, however, its function and mechanism of pathogenesis remain unclear.

**Research frontiers**

The pathogenesis of _H. pylori_ is partially clear, as _H. pylori_ may secrete many types of toxins. With the exception of CagA and VacA, new _H. pylori_ toxins have been discovered, such as Tip-α, mammalian, prokaryotic high-temperature requirement A, and the duodenal ulcer-promoting gene. Only the function of the toxins and their mechanism of pathogenesis are clear, as the mechanism of _H. pylori_ pathogenesis is known.

**Innovations and breakthroughs**

Other studies discovered that Tip-α promoted the expression of cytokines, however, this article first showed the difference in the promotion of the expression of cytokines between gastric epithelial cells and cancer cells, and that Tip-α activates cytokine expression in a nuclear factor κB (NF-κB)-dependent manner.

**Applications**

Tip-α may become a marker of _H. pylori_ virulence. The virulence of _H. pylori_ may be determined by detecting Tip-α.

**Terminology**

Tip-α is the abbreviation for tumor necrosis factor-α inducing protein. It was first discovered that this new _H. pylori_ toxin can promote the expression of TNF-α, therefore, it was called Tip-α.

**Peer review**

The authors certified that Tip-α, a new toxin of _H. pylori_, promoted the expression of cytokine, discovered the difference of this function between gastric epithelial cells and cancer cells, and in an NF-κB-dependent manner, further interpreted the mechanism of pathogenesis of _H. pylori_.

**REFERENCES**

1. Nomura A, Stemmermann GN, Chyou PH, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and the risk for duodenal and gastric ulceration. _Ann Intern Med_ 1994; 120: 977-981 [PMID: 7741826]

2. Kuipers EF. Helicobacter pylori and the risk and management of associated diseases: gastritis, ulcer disease, atrophic gastritis and gastric cancer. _Aliment Pharmacol Ther_ 1997; 11 Suppl 1: 71-88 [PMID: 9146793 DOI: 10.1046/j.1365-2036.11.s1.15.x]

3. Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, Orentreich N, Vogelman JH, Friedman GD. Helicobacter pylori infection and gastric lymphoma. _N Engl J Med_ 1994; 330: 1267-1271 [PMID: 8145781 DOI: 10.1056/NEJM199405053301803]

4. Johannson H. Qualification conditions: invest more firmly in continuing education. _Vardfacket_ 1978; 2: 28-30 [PMID: 249197 DOI: 10.1002/1097-0142(19901215)66:22328-30 [PMID: 8145781 DOI: 10.1056/NEJM199012153301803]

5. Müller H, Heselstein E, Vainio H. Working group report on schistosomes, liver flukes and Helicobacter pylori. _Int J Cancer_ 1995; 60: 587-589 [PMID: 7860130 DOI: 10.1002/ijc.2901600502]

6. Segal ED, Tompkins LS. Identification and characterization of a Helicobacter pylori hemolysin. _Infect Agents Dis_ 1993; 2: 178-182 [PMID: 8173790]

7. Smoliany BL, Piotrowski J, Sengupta S, Smoliany A. Inhibition of gastric mucosal laminin receptor by Helicobacter pylori lipopolysaccharide. _Biochem Biophys Res Commun_ 1991; 175: 963-970 [PMID: 1822728 DOI: 10.1016/0006-291X(91)91569-Z]

8. Leunk RD, Johnson PT, David BC, Kraft WG, Morgan DR. Cytotoxic activity in broth-culture filtrates of Campylobacter pylori. _J Med Microbiol_ 1988; 26: 93-99 [PMID: 3385767 DOI: 10.1099/0022615-2-2-93]

9. Censini S, Lange C, Xiang Z, Crabtree JE, Ghiaa P, Borodovsky M, Rappaport R, Covacci A. cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. _Proc Natl Acad Sci USA_ 1996; 93: 14648-14653 [PMID: 9862108 DOI: 10.1073/pnas.93.25.14648]

10. Crabtree JE, Court M, Aboshkiva MA, Jeremy AH, Dixon MF, Robinson PA. Gastric mucosal cytokine and epithelial cell responses to Helicobacter pylori infection in Mongolian gerbils. _J Pathol_ 2004; 202: 197-207 [PMID: 14743502 DOI: 10.1002/path.1498]

11. Fan X, Chua A, O’Connell MA, Kelleher D, Keeling PW. Interferon-gamma and tumour necrosis factor factor production in patients with Helicobacter pylori infection. _J Med Sci_ 1993; 162: 408-411 [PMID: 8300378 DOI: 10.1007/BF02963139]

12. Huang J, O’Toole PW, Doig P, Trust TJ. Stimulation of interleukin-8 production in epithelial cell lines by Helicobacter pylori. _Infect Immun_ 1995; 63: 1732-1738 [PMID: 7729879]

13. Noach LA, Bosma NB, Jansen J, Hoek FJ, van Deventer SJ, Tytgat GN. Mucosal tumor necrosis factor-alpha, interleu-
kin-1 beta, and interleukin-8 production in patients with Helicobacter pylori infection. *Scand J Gastroenterol* 1994; 29: 425-429 [PMID: 8036458 DOI: 10.3109/00365529409096833]

14 **Gionchetti P**, Vaira D, Campieri M, Holton J, Menegatti M, Belluzzi A, Bertinelli E, Ferretti M, Brigona C, Miglioli M. Enhanced mucosal interleukin-6 and -8 in Helicobacter pylori-positive dyspeptic patients. *Am J Gastroenterol* 1994; 89: 883-887 [PMID: 8198099]

15 **Suganuma M**, Kurusu M, Suzuki K, Nishizono A, Murakami K, Fujioka T, Fujiki H. New tumor necrosis factor-alpha-inducing protein released from Helicobacter pylori for gastric cancer progression. *J Cancer Res Clin Oncol* 2005; 131: 305-313 [PMID: 15616827 DOI: 10.1007/s00432-004-0652-x]

16 **Godlew ska R**, Pawłowski M, Dzwonek A, Mikula M, Ostrowski J, Drela N, Jagusztyn-Krynicka EK. Tip-alpha (hp0596 gene product) is a highly immunogenic Helicobacter pylori protein involved in colonization of mouse gastric mucosa. *Curr Microbiol* 2008; 56: 279-286 [PMID: 18172719 DOI: 10.1007/s00284-007-9083-7]

17 **Tosi T**, Cioci G, Jouravleva K, Dian C, Terradot L. Structures of the tumor necrosis factor a inducing protein Tipa: A novel virulence factor from Helicobacter pylori. *FEBS Letters* 2009; 583: 1581-1585 [DOI: 10.1016/j.febslet.2009.04.033]

18 **Kuzuhara T**, Suganuma M, Kurusu M, Fujiki H. Helicobacter pylori-secreted protein Tipalpha is a potent inducer of chemokine gene expressions in stomach cancer cells. *J Cancer Res Clin Oncol* 2007; 133: 287-296 [PMID: 17393199 DOI: 10.1007/s00432-006-0169-6]

19 **Rossi D**, Zlotnik A. The biology of chemokines and their receptors. *Annu Rev Immunol* 2000; 18: 217-242 [PMID: 10837058 DOI: 10.1146/annurev.immunol.18.1.217]

20 **Massari P**, Manetti R, Burroni D, Nuti S, Norais N, Rappuoli R, Telford JL. Binding of the Helicobacter pylori vacuolating cytotoxin to target cells. *Infect Immun* 1998; 66: 3981-3984 [PMID: 9673292]

21 **Odenbreit S**, Püls J, Sedlmairer B, Gerland E, Fischer W, Haas R. Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. *Science* 2000; 287: 1497-1500 [PMID: 10688880 DOI: 10.1126/science.287.5457.1497]

22 **Suganuma M**, Yamaguchi K, Ono Y, Matsumoto H, Hayashi T, Ogawa T, Imai K, Kuzuhara T, Nishizono A, Fujiki H. TNF-alpha-inducing protein, a carcinogenic factor secreted from H. pylori, enters gastric cancer cells. *Int J Cancer* 2008; 123: 117-122 [PMID: 18412243 DOI: 10.1002/ijc.23484]

23 **Watanabe T**, Tsuge H, Imagawa T, Kise D, Hirano K, Beppu M, Takahashi A, Yamaguchi K, Fujiki H, Suganuma M. Nucleolin as cell surface receptor for tumor necrosis factor-alpha inducing protein: a carcinogenic factor of Helicobacter pylori. *J Cancer Res Clin Oncol* 2010; 136: 911-921 [PMID: 20049476 DOI: 10.1007/s00432-009-0733-y]

24 **Kuzuhara T**, Suganuma M, Oka K, Fujiki H. DNA-binding activity of TNF-alpha inducing protein from Helicobacter pylori. *Biochem Biophys Res Commun* 2007; 362: 805-810 [PMID: 17765875 DOI: 10.1016/j.bbrc.2007.05.181]

25 **Scheer R**, Meier B, Männel DN, Dröge W, Baueuerle PA. Di-thiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells. *J Exp Med* 1992; 175: 1181-1194 [PMID: 1314883]

26 **Suganuma M**, Kuzuhara T, Yamaguchi K, Fujiki H. Carcinogenic role of tumor necrosis factor-alpha inducing protein of Helicobacter pylori in human stomach. *J Biochem Mol Biol* 2006; 39: 1-8 [PMID: 16466631]