

ANSWERING REVIEWERS

Reviewer's code: 03408355

Reviewer's country: China

Dear reviewer,

first of all, we are grateful for the very important comments, which were essential to the improvement of this manuscript. All suggested changes were made as described below:

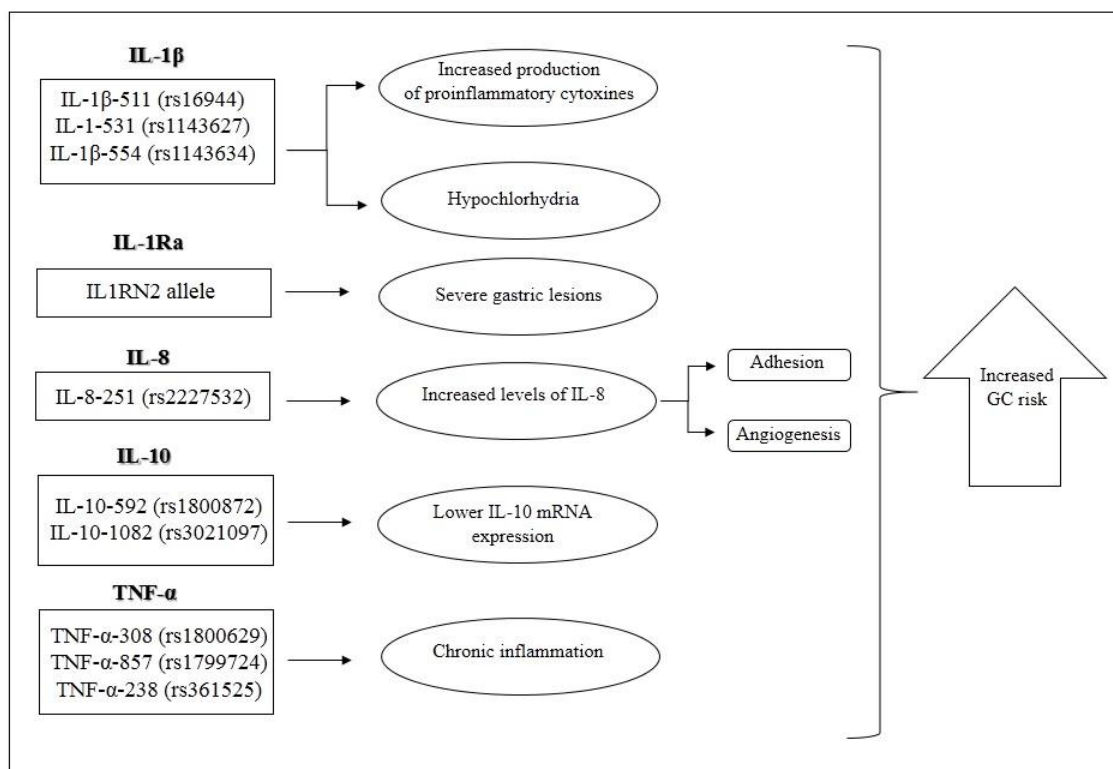
1. The abstract was revised to be more specific and informative.

Abstract

The *Helicobacter pylori* infection is a determinant factor in gastric cancer (GC) development. However, the infection outcomes are variable and depend on both host and bacterial characteristics. Some host cytokines such as interleukin (IL)-1 β , IL-1Ra, IL-8, IL-10 and tumor necrosis factor (TNF)- α play important role in the host immune system response to the pathogen, in the development of gastric mucosal lesions and in cell malignant transformation. Therefore, these host factors are crucial in neoplastic processes unleash. Certain polymorphisms in genes that encode these cytokines have been associated with an increased risk of GC. On the other hand, various virulence factors found in distinct *H. pylori* bacterial strains, including cytotoxin-associated antigen A (CagA), vacuolating cytotoxin (VacA), duodenal ulcer promoting gene A protein (DupA), outer inflammatory protein (OipA) and blood group antigen binding adhesin (BabA), have been associated with the pathogenesis of different gastric diseases. The virulent factors mentioned above allow the successful infection by the bacterium and play crucial role in gastric mucosa lesions, including malignant transformation. Moreover, the role of host polymorphisms and bacterial virulence factors in gastric carcinogenesis seems to vary among different countries and populations. The identification of host and bacterium factors that are associated with an increased risk of GC development may be useful in determining the prognosis of infection in patients, what could help in clinical decision-making and in the providing of an optimized clinical approach.

2. The subtitles were enumerated as suggested.

3. A figure illustrating the potential function of the genetic polymorphisms in gastric carcinogenesis was drawn:



4. The references were updated and 13 references were replaced by recent publications.

DELETED REFERENCES:

a) **Hojsak I**, Kolacek S. Is *Helicobacter pylori* always a "bad guy"? [PMID: 24180409]

b) **International Agency for Research on Cancer**. IARC monographs on the evaluation of the carcinogenic risks to humans. Schistosomes, liver flukes and *Helicobacter pylori*. Lyon: International Agency for Research on Cancer, 1994; 61: 177

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d) **Odenbreit S**, Püls J, Sedlmaier 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: 10688800 DOI: 10.1126/science.287.5457.1497]

- e) **Ito R**, Kitadai Y, Kyo E, Yokozaki H, Yasui W, Yamashita U, Nikai H, Tahara E. Interleukin-1-Alpha Acts as an Autocrine Growth Stimulator for Human Gastric- Carcinoma Cells. *Cancer research*. 1993; 53(17): 4102-6. [PMID: 8358739]
- f) **Rozengurt E**, Walsh JH.. Gastrin, CCK, signaling, and cancer. *Annu Rev Physiol*. 2001;63:49–76.[PMID:11181948 DOI: 10.1146/annurev.physiol.63.1.49]
- g) **El-Omar EM**, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF Jr, Rabkin CS. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000;404:398–402. [PMID: 10746728 DOI: 10.1038/35006081]
- h) **Taub DD**, Oppenheim JJ. Chemokines, inflammation and the immune system. *Ther Immunol*. 1994;1(4):229–246.[PMID: 7584498]
- i) **Y Kitadai**, Y Takahashi, K Haruma, K Naka, K Sumii, H Yokozaki, W Yasui, N Mukaida, Y Ohmoto, G Kajiyama, I J Fidler, E Tahara. Transfection of interleukin-8 increases angiogenesis and tumorigenesis of human gastric carcinoma cells in nude mice. *Br J Cancer*. 1999;81(4):647–653.[doi: 10.1038/sj.bjc.6690742 PMID: 10574250]
- j) **Hull J**, Thomson A, Kwiatkowski D. Association of respiratory syncytial virus bronchiolitis with the interleukin 8 gene region in UK families. *Thorax*. 2000;55(12):1023–1027. [PMID: 11083887 PMCID: PMC1745668]

- k) Locksley, R. M., Killeen, N., & Lenardo, M. J. (2001).** The TNF and TNF receptor superfamilies: Integrating mammalian biology. *Cell*, 104, 487–501. [PMID: 11239407]

- l) Telford JL, Ghiara P, Dell'Orco M, Comanducci M, Burroni D, Bugnoli M, Tecce MF, Censini S, Covacci A, Xiang Z,.** Gene structure of the *Helicobacter pylori* cytotoxin and evidence of its key role in gastric disease. *J Exp Med* 1994; 179: 1653–1658.[PMID: 8163943 PMCID: PMC2191472]

- m) Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Borén T.** *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. *Science*, v. 279, p. 373-377, 1998. [PMID: 9430586]

NEW REFERENCES:

- a) Alzahrani S, Lina TT, Gonzalez J, Pinchuk IV, Beswick EJ, Reyes VE.** Effect of *Helicobacter pylori* on gastric epithelial cells. *World J Gastroenterol* 2014; **20**: 12767-12780 [PMID: 25278677 DOI: 10.3748/wjg.v20.i36.12767]

- b) Shimizu T, Marusawa H, Watanabe N, Chiba T.** Molecular Pathogenesis of *Helicobacter pylori*-Related Gastric Cancer. *Gastroenterol Clin North Am* 2015; **44**: 625-638 [PMID: 26314672 DOI: 10.1016/j.gtc.2015.05.011]

- c) Ang TL, Fock KM.** Clinical epidemiology of gastric cancer. *Singapore Med J* 2014. **55**: 621-628 [PMID: 25630323]

- d) Wang F, Meng W, Wang B, Qiao L.** *Helicobacter pylori*-induced gastric inflammation and gastric cancer. *Cancer Lett* 2014. **345**: 196-202 [PMID: 23981572 DOI: 10.1016/j.canlet.2013.08.016]

- e) Ma J, Sawai H, Matsuo Y, Ochi N, Yasuda A, Takahashi H, Wakasugi T, Funahashi H, Sato M, Okada Y, Takeyama H, Manabe T.** Interleukin-1alpha enhances angiogenesis and is associated with liver metastatic potential in human gastric cancer cell lines. *J Surg Res* 2008. **148**: 197-204 [PMID: 18395750 DOI: 10.1016/j.jss.2007.08.014]

- f) Huang FY, Chan AO, Lo RC, Rashid A, Wong DK, Cho CH, Lai CL, Yuen MF.** Characterization of interleukin-1 β in *Helicobacter pylori*-induced gastric inflammation and DNA methylation in interleukin-1 receptor type 1

knockout (IL-1R1(-/-)) mice. *Eur J Cancer* 2013. **49**: 2760-2770 [PMID: 23664095 DOI: 10.1016/j.ejca.2013.03.031]

- g) **Chiurillo MA.** Role of gene polymorphisms in gastric cancer and its precursor lesions: current knowledge and perspectives in Latin American countries. *World J Gastroenterol* 2014. **20**: 4503-4515 [PMID: 24782603 DOI: 10.3748/wjg.v20.i16.4503]
- h) **Sun Q,** Sun F, Wang B, Liu S, Niu W, Liu E, Peng C, Wang J, Gao H, Liang B, Niu Z, Zou X, Niu J. Interleukin-8 promotes cell migration through integrin $\alpha\beta 6$ upregulation in colorectal cancer. *Cancer Lett* 2014. **354**: 245-253 [PMID: 25150782 DOI: 10.1016/j.canlet.2014.08.021]
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- j) **Vairaktaris E,** Yapijakis C, Serefoglou Z, Derka S, Vassiliou S, Nkenke E, Vylliotis A, Wiltfang J, Avgoustidis D, Critselis E, Neukam FW, Patsouris E. The interleukin-8 (-251A/T) polymorphism is associated with increased risk for oral squamous cell carcinoma. *Eur J Surg Oncol* 2007. **33**: 504-507. [PMID: 17174061 DOI: 10.1016/j.ejso.2006.11.002]
- k) **Croft M,** Benedict CA, Ware CF. Clinical targeting of the TNF and TNFR superfamilies. *Nat Rev Drug Discov* 2013. **12**:147-168 [PMID: 23334208 PMCID: PMC3625401 DOI: 10.1038/nrd3930]
- l) **Isomoto H,** Moss J, Hirayama T. Pleiotropic actions of Helicobacter pylori vacuolating cytotoxin, VacA. *Tohoku J Exp Med* 2010. **220**: 3-14 [PMID: 20046046]
- m) **Shahi H,** Reisi S, Sadeghiani M, Mahsa M, Bahreini R, Moghni M, damavandi M, fatollahi F, Shahverdi E , ramezani G , Shirzad H. Prevalence of cagA and babA2 genes in Helicobacter Pylori strains Isolated from Iranian gastrointestinal disorder patients and their gastritis classification. *J. Biol. Today's World* 2014. **3**: 256-260 [DOI:10.15412/J.JBTW.01031201]

Best regards,

Fabrício Freire de Melo

Professor, PhD

Reviewer's code: 00505440

Reviewer's country: Australia

Dear reviewer,

the comment you made was useful and we are grateful for your crucial help. Your comment was very important to the language polish of this manuscript. The correction of the spelling of 'Cytotoxin' from 'Citotoxine' was made as suggested.

Best regards,

Fabrício Freire de Melo

Professor, PhD

Reviewer's code: 01799104

Reviewer's country: Taiwan

Dear reviewer,

thank you for the crucial comment about this manuscript. Your suggestion was important for its formatting adequacy. The references were formatted with the purpose of becoming uniform as suggested:

1. **Alzahrani S**, Lina TT, Gonzalez J, Pinchuk IV, Beswick EJ, Reyes VE. Effect of *Helicobacter pylori* on gastric epithelial cells. *World J Gastroenterol* 2014; **20**: 12767-12780 [PMID: 25278677 DOI: 10.3748/wjg.v20.i36.12767]
 2. **Shimizu T**, Marusawa H, Watanabe N, Chiba T. Molecular Pathogenesis of *Helicobacter pylori*-Related Gastric Cancer. *Gastroenterol Clin North Am* 2015; **44**: 625-638 [PMID: 26314672 DOI: 10.1016/j.gtc.2015.05.011]
 3. **Ang TL**, Fock KM. Clinical epidemiology of gastric cancer. *Singapore Med J* 2014; **55**: 621-628 [PMID: 25630323]
 4. **Wang MY**, Liu XF, Gao XZ. *Helicobacter pylori* virulence factors in development of gastric carcinoma. *Future Microbiol* 2015; **10**: 1505-1516 [PMID: 26346770 DOI: 10.2217/fmb.15.72]
- [...]

81. Talebi Bezmin Abadi A, Taghvaei T, Mohabbati Mobarez A, Vaira G, Vaira D. High correlation of babA 2-positive strains of *Helicobacter pylori* with the presence of gastric cancer. *Intern Emerg Med* 2013. **8**: 497-501. [PMID: 21604199 DOI: 10.1007/s11739-011-0631-6]

Best regards,

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