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The Progress in *Helicobacter Pylori* High Risk Pathogenic Markers

Wang L[1], **Zhang J**[1], **Shi YY**[1], **Lin SR**[1] **and Ding SG**[1*]

[1]*Gastrointestinal Department, Peking University Third Hospital, Beijing, China*

[*]**Corresponding author:** DING Shigang,Gastrointestinal Department, Peking University Third Hospital, Beijing, China, E-mail: dingshigang222@126.com

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Abstract

It has been widely accepted that *Helicobacter pylori* was closed related with tumorigenesis of gastric cancer, but the pathogenic factor of this bacteria as well as the intrinsic mechanism are still unclear. We analyzed the proteomics of *Helicobacter pylori* strains isolated from both gastritis and gastric cancer and discovered several differential proteins. Among these proteins the Thioredoxin-1 (Trx-1) was considered most significant. Trx-1 protein has an anti-oxidative function and might increase cellular proliferation and anti-apoptosis. It helped to protect *Helicobacter pylori* from the oxidative reaction from the host so as lead to the long term colonization. We carried out a series of researches on this protein. The results revealed that Trx-1 was highly expressed in *Helicobacter pylori* isolated from cancer patients compared with the bacteria from gastritis patients. In cell culture study, up-regulation of Trx-1 expression in GES-1 and BCG823 cell lines might increase cell growth and promote cells into S phase. What's more, infected with *Helicobacter pylori* that express high level Trx-1 might induced cell apoptosis, decreased the expression of cyclin D1 and upregulated p21 in GES-1 cell line, while increase cell proliferation, and upregulate cyclin D1 in BCG823 cell line, indicating an oncogenic effects. We further infected Mongolian gerbils by *Helicobacter pylori* with high level Trx-1. The results showed long term infection lead to serious pathologic change of the stomach mucosa and finally adenocarcinoma occurred. In conclusion, Helicobacter pylori Trx-1 may play an important role in the development of stomach adenocarcinoma and can be considered as pathogenic marker. Future studies are still necessary for the specific process of Trx-1 protein on gastric mucosal after *H. pylori* infection, the relationship between clinical TNM stages and Hp Trx-1 level, as well as the downstream signal pathways that are involved in the carcinogenic process.

Introduction

In 1983 the bacteria Helicobacter pylori (*H. pylori*) was first isolated by Warren and Marshall. *H. pylori* is widely spreading in the world and approximately 50% human populations are chronically infected. Nowadays it has been accepted that infection with H. Pylori is strongly associated with chronic active gastritis, peptic ulcer, gastric cancer and MALT Lymphoma. In 1994, *H. pylori* was classified as a group I carcinogen by WHO. Anyway, only a subpopulation of the infected patients develops serious clinical consequences [1]. As is known to all, the bacterial pathogenic factor and host genetic liability combine to determine the degree of gastric damage. Previous researches focused a lot on human genetic factors and several relevant genes had been confirmed, such as IL-1, TNF-α, etc. (reviewed by Shanks et al. [2]). Anyway, few novel bacterial factors have been determined in the past years. Therefore, to identify the relevant *H. pylori* pathogenic factors are becoming increasingly important, that patients infected with high pathogenic *H. pylori* should receive therapeutic intervention.

Trx-1 was highly expressed in *H. pylori* isolated from gastric cancer patients

To date several risk factors of *H. pylori* have been investigated. CagA and VacA are two risk factors that are mostly noticed in western countries [3]. Anyway, in China nearly 90% *H. pylori* are CagA positive but only approximately 1% infected individuals finally might develop gastric cancer [4]. Thus lot of other factors is attracting more attention. Previously our team analyzed the proteomic differences of *H. pylori* strains isolated from gastric cancer and gastritis and found several different expressed proteins [5-7]. Among these proteins the Thioredoxin-1 (Trx-1) was considered most significant. Based on current studies we focus on this interesting protein and carried out a series of researches including cell lines, animal study, human tissue as well as functional analysis of this protein, and the results showed that Trx-1 might be an important pathogenic factor of *H. pylori*.

The factor Trx was first isolated by Laurent in 1964 [8]. In mammals two types of Trx has been isolated including Trx-1 and Trx-2, which located in different subcellular areas. Trx and its receptor contributed to the oxidation - reduction system of cell, which was very important in defensing oxidative stress and maintaining cellular survival. Previous experiments showed that human Trx-1 played roles in promoting cell proliferation and anti-apoptosis process [9]. In 2006 Zhang et al [7] used proteomic method to analyze *H. pylori* strains isolated from gastric cancer, peptic ulcer and gastritis patients.

The result showed that Trx-1 level was significantly higher in *H. pylori* from cancer patients than that from other groups. Considering the inflammation process after *H. pylori* infection as well as the oxidative stress by host could lead to a series pathogenic change and finally related with tumorigenesis, we supposed that the high pathogenic *H. pylori* strains might have different mechanism in carcinogenesis, the factors anticipating the anti-oxidative mechanism would promote this process, and Trx-1 might be closely involved in this complex procedure and act as *H. pylori* pathogenic factor. Therefore, we carried out a series of experiments aiming to clarify the mechanism as well as to determine the risk factors in *H. pylori*.

Functional analysis of Trx-1

In order to clarify the possible mechanism of Trx-1 in the carcinogenic process, we further carried out a series of studies including the influence of *H. pylori* and the Trx-1 protein on cell culture and the downstream signal transduction, the outcome of animals infected by *H. pylori* with different Trx-1 levels, and also the Trx-1 conditions in human tissue from different diseases, which will be described in detail.

Previous research revealed that Trx-1 protein in human might increase cellular proliferation and anti-apoptosis function [10]. To construct the system, we successfully cloned the human Trx-1 gene and obtained the recombinant vector containing the target gene [11]. We also sequenced the two subtypes of *H. pylori* Trx-1 and Trx-2 gene, and confirmed a redox active motif Cys-Gly-Pro-Cys in Trx-1 gene, which was closed related with its anti-oxidative function [12]. What's more, we obtained human Trx-1 gene sequence and reconstructed human Trx-1 protein with biological activity. Then *H. pylori* Trx-1 was overexpressed in gastric cancer cell line BCG823 and gastric epithelial cell line GES-1 by transfection, and the result revealed that up-regulation of Trx-1 might significantly increase cell growth and promote cells into S phase [13]. We also infected these two cell lines with *H. pylori* of high Trx-1 level and further confirmed the carcinogenic ability of high Trx-1 *H. pylori*. In gastric mucosal epithelial cell line GES-1, infected by *H. pylori* with high Trx-1 level might significantly induced cell apoptosis, decreased the expression of cyclin D1 and upregulated p21, which indicated a stronger ability to damage the gastric mucosal epithelium and pathogenicity. Anyway, in gastric cancer cell line BGC823, high Trx-1 H. pylori might significantly increase cell proliferation, and upregulate cyclin D1, which indicated the oncogenic effects [14].

Long term infection of *H. pylori* would lead to malignant change of gastric mucosa. To further clarify the tumorigenic ability of *H. pylori* with high Trx-1 expression, we infected the SPF Mongolian gerbils and observed histologic and molecular change. In high Trx-1 *H. pylori* infection group, the stomach mucosa showed earlier and more obviously aggravation change over time and finally adenocarcinoma occurred [15]. This in vivo experiment further confirmed that *H. pylori* with high level Trx-1 had stronger tumorigenic ability, and Trx-1 might be considered as a pathogenic marker of *H. pylori*.

Relationship between Trx-1 and other antioxidant members

Previous research confirmed that *H. pylori* Trx1 was a secretory protein which was secreted by type IV secretion system when the bacteria was subjected to stress stimuli, such as chemical, biological and environmental stimuli. It was speculated that in the colonization process of *H. pylori* to the host, Trx1 could decrease the reaction of the host by catalyzing the reduction reaction, so as to help the colonization process. But the specific mechanisms are still under investigation. Interestingly, in our experiment on human endoscope biopsy tissue, the *H. pylori* Trx-1 protein level was also found higher in the cancer tissue than in gastritis mucosa [16], which prompted the need for further analysis of Trx-1 levels in human tissue from different diseases, as well as the underling mechanism for the difference.

Previously our data also revealed another two anti-oxidative factors, the arginase (RocF) and alkyl-hydroperoxide reductase (AhpC) levels were also higher in *H. pylori* isolated from gastric cancer patients [17]. RocF is also an important part of *H. pylori* redox system as Trx-1. Physiological function of RocF is to catalyze the hydrolysis of l-arginine to generate l-ornithine and urea. It also plays part in the evasion of the host's immune system and thus contributes to persistent infection [18]. RocF negative *H. pylori* infection of GES-1 cell line would lead to an increase of IL-8, indicating the intrinsic modulation of RocF on host inflammatory cell signals [19]. AphC is an enzyme that reduces peroxides and may protect bacterial against reactive nitrogen intermediates [20]. It is also remarkable that the levels of Trx-1 and RocF in *H. pylori* strains were closely related. The RocF level was also higher in *H. pylori* isolated from cancer patients. What's more, our data showed a positive correlation trend between Trx-1 and AphC, although there was no linear trend, it might due to the limited sample number [17]. So we might come to the conclusion that it is the antioxidant enzyme system including Trx-1, RocF and AphC that contribute to the long term colonization of *H. pylori* in host stomach, and avoid *H. pylori* from oxidative damage. Proteins participating in this system might be intrinsic tumorigenic factors as well as clinical markers to determine high risk *H. pylori* strains.

Further study

In conclusion, *H. pylori* with high level Trx-1 expression might cause more serious pathologic changes during long term infection. *H. pylori* Trx-1 may play an important role in the development of stomach adenocarcinoma. Anyway, there are still a lot of uncertain points to be investigated. Future studies will be focused on the specific process of Trx-1 protein on gastric mucosal after *H. pylori* infection, as well as the downstream signal pathways that involved in the carcinogenic process. In clinical study, the relationship between Hp Trx-1 level and patients TNM stages would be further analyzed. Besides, clarifying the mRNA levels of these markers in human tissue would help rapidly screening of high-risk individuals, which might be very important in clinical works.

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