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Fur: As a Potential Killer of H. Pylori Iron Death in the Stomach

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Abstract: ***Helicobacter pylori (H. pylori) is the first pathogenic bacterium found in the stomach causing maltiple diseases related to the digestive system. Studies on H. pylori are important for understanding the overall population of the bacteria that reside in the digestive tract. Ferric uptake regulation protein (Fur) excellently regulates H. pylori and is closely related to the structure, pathogenesis, escape, and transfer mechanisms of the bacterium. Focusing on the correlation between Fur and H. pylori, the involvement of Fur in different aspects of H. pylori in host organisms was systematically investigated. Our research revealed that Fur is involved in H. pylori colonization in the stomach, altering the gastric environment to create more suitable survival conditions, inducing pathogeny of the host, and curing H. pylori-related diseases. Fur has an important role in the colonization of H. pylori, and Fur appears to be influenced by a variety of trace elements, including iron, and altering iron homeostasis appears to be an effective means of inhibiting Fur. In addition to the factor that H. pylori and the host belong to two species, the strong acidic environment in the stomach also acts as a catalyst for chemical reactions that create favorable factors for the altered iron homeostasis environment. The performance of Fur makes it impossible to ignore the presence of Fur in the study of H. pylori, and the induction of iron death by creating a homeostatic environment in the stomach that inhibits Fur may become a new research direction and play an important role in the treatment of H. pylori. Moreover, Fur has a significant impact on the progression of various digestive diseases.***

Keywords: *Helicobacter pylori*, Fur, Colonization, Atrophic gastritis, Iron deficiency anemia**.**

1. Introduction

Chronic atrophic gastritis (CAG) is a common digestive disorder characterized by bloating, abdominal pain, acid reflux, weight loss, and secondary anemia [1]. CAG is also globally recognized as a precancerous lesion with a high risk of transformation to gastric cancer [2]. Iron plays a critical role in the development and progression of numerous malignancies. Different epidemiological studies have highlighted a link between an enhanced risk of gastrointestinal cancer and iron deficiency anemia (IDA) [3,4]. H. pylori is the most important infectious agent in cancer etiology. Studies have shown that H. pylori-infected individuals have more intense CAG and exhibit a much enhanced risk of developing gastric cancer than healthy individuals [5,6]. As H. pylori can adapt to the extremely acidic environment of the stomach, it colonizes the host causing persistent infection. This ultimately leads to an aggravation of CAG and the consequent development of cancers [7]. Ferric uptake regulation protein (Fur) regulates H. pylori colonization and survival in the gastric mucosa [8]. Moreover, H. pylori Fur regulates gene expression in metal-binding as well as metal-free (apo-Fur) forms [9]. Fur has demonstrated excellent roles in multiple aspects of H. pylori regulation and appears to be related to its colonization, escape mechanisms, oxidative stress, and therapy. Starting from the characteristics of Fur seems to be able to be a new direction for the treatment of H. pylori, while the strong acidic environment in the stomach has an important role in driving the chemical reaction of displacing metal ions. This study focuses on the Fur-H. pylori relationship and discusses the significant role of Fur in H. pylori colonization in the stomach, post-colonization changes in the gastric environment to create more suitable conditions for survival, induction of pathogenesis of host, treatment of H. pylori-related diseases, The possibility of building an environment that inhibits Fur iron homeostasis in the stomach was also analyzed, and the hypothesis that the creation of differential concentrations of trace elements in the same environment for different species leads to the occurrence of iron death was proposed, and provides new strategies for H. pylori treatment targeting Fur.

2. Biochemical Characteristics of Fur

Fur is a transcription factor that utilizes Ferrous iron (Fe^{2+}) as a corepressor and inhibits iron carrier synthesis in pathogens. Fe2+ is one of the earliest metal cofactors [10]. Fur regulates directly or indirectly the expression of enzymes which can inhibit reactive oxygen species (ROS). Therefore, the challenge of iron homeostasis and defense against ROS is addressed by Fur [11].

The Fur of H. pylori differs from the Furs in other bacteria because it is an atypical variety containing three distinct metal binding sites (S1, S2, and S3) [12]. S1 is a tetrahedral ZnS4 structural site that stabilizes the β3-β4-β5 sheet, a key element in the dimer formation of H. pylori Fur. S2 is essential for the DNA binding of H. pylori Fur, and metallization of S2 triggers conformational changes in H. pylori Fur. The S3 site significantly reduces the DNA binding affinity of H. pylori Fur. This suggests that metal ion binding at S3 enhances the DNA-binding affinity of H. pylori Fur for additional metallic DNA [12].

Iron is an essential element for organisms. Its enzymes mediate a variety of physiological functions [13], including cellular metabolism and respiration, transport of oxygen, synthesis of DNA, and production of energy. In addition, the catalytic form of iron catalyzes ROS formation in oxygen-rich environments.

Fur can be involved in acquiring several metals, including ferric ions [14]. When iron availability is high, Fur functions as a repressor. Using Fe2+ as a cofactor [15], it binds to the

promoter regions of several genes to negatively regulate their transcription [16,17]. The Fur family of metal regulators includes ferric (Fur), zinc (Zur), manganese (Mur), nickel (Nur), and peroxide stress (PerR) sensors [18]. However, other metal regulatory functions are not encoded in H. pylori. Therefore, Fur is vital for organisms to adapt to multiple stresses caused by acid, iron, and ROS [19].

3. Fur Actively Promotes and Effectively Limits H. Pylori Colonization

Fur movement of H. pylori is crucial for the initial establishment of a bacterial colony. Fur acts as a regulator and is involved in the process of ferric uptake and colonization of H. pylori [20]. H. pylori can colonize in extreme environments within the stomach and influence the body to exacerbate the progression of multiple diseases. Fur is involved in the inhibition of gastric acid production and resistance to ROS, thus building an adaptive environment for H. pylori. Thus, Fur promotes H. pylori colonization by participating in and influencing the aforementioned processes [21,22]. Fur regulates various genes that contribute to H. pylori colonization in the organism, such as FEC A1 and FeoB that regulate iron transport [23,24] nd putA that regulates Proline metabolism [25]. Chelsey R. Fontenot et al. found that H. pylori Fur binds the [2Fe-2S] cluster in response to the raised level of intracellular free iron [26]. The crucial role of Fur in adapting to an acidic environment might be partially related to the regulation of amide E and amiF, by which the amidases degrade amides to synthesize ammonia [27]. Additionally, it might also be related to NikR, which directly regulates the accessibility of trace elements such as nickel along with iron availability in cells and Fur expression in response to acidic environments [28,29].

The regulation of H. pylori by Fur is particularly precise. Research shows that Fur not only assists in H. pylori colonization but also controls it. Evidence of H. pylori colonization at the gastric sinus tends to be higher than at the gastric body, which seems to correlate with gastric acid secretion [30,31]. Fur regulates the expression of the gene encoding the chemotactic protein CheV2 [32], which seems to be associated with atypical colonization of H. pylori [33].

4. Fur and Its Related Substances Can Effectively Inhibit Oxidative Stress

The H. pylori colonizing the stomach can express abundant antioxidant factors and enzymes to neutralize oxidative stimuli that are detrimental to its colonization [34]. The studies of ROS in low levels of metronidazole or oxygen reveal that H. pylori probably has some unique defense mechanisms [35], which show a close correlation between the regulation of this oxidative stress defense and Fur [36]. The role of Fur in redox homeostasis is not surprising, as the formation of hydroxyl radical species is closely related to the availability of free ions of intracellular iron generated via the Fenton reaction [37]. In addition, the superfamily of Fur regulators includes PerR and other direct homologs that play important roles in oxidative stress signaling in a variety of bacteria [38,39]. The variant regulation of Fur is able to transduce H. pylori in oxidative stress signaling, in line with the idea that iron-induced apo-Fur repressor genes can also act as targets of oxidation-induced Fur regulation [40].

5. Fur affects the Survival of Other Flora in the Stomach

The typical gastric microbiota is composed of 57 bacterial genera distributed in eight species, including Actinobacteria, Bacteroides, Spirochetes, Firmicutes, Clostridia, Proteobacteria, Gardnerella, and TM7 [41,42]. H. pylori is well adapted to the extremely acidic environment of the stomach. While in the stomach, it is the most abundant organism in the gastric microbiota, accounting for 40–90% of the population [43]. A considerable negative relationship exists between H. pylori and other bacteria in the stomach. This negative correlation serves as additional evidence that H. pylori might affect the survival of other bacteria in the stomach [44-46]. Further experiments exploring the correlation revealed that the presence or absence of H. pylori would change the bacteria in the stomach. Individuals having H. pylori will exhibit a higher abundance of B. Albicans and a lower abundance of Actinobacteria, Bacteroides, and Firmicutes [47,48]. Fur plays a crucial role in iron uptake by a variety of intragastric bacteria, and multiple uptake mechanisms guarantee that adequate iron is available for the uptake [49]. Colonized H. pylori regulate ferric uptake via Fur, which further alters the acid environment of the stomach, thereby altering the colonization of the intragastric flora [50, 51]. Studies have shown that microorganisms, including Escherichia coli, Lactobacillus spp., Nitrospira spp., Veillonella spp., Haemophilus spp., Clostridium Bacillus spp., and Staphylococcus spp. promote cancer development by converting nitrogen compounds in gastric juice to potentially carcinogenic N-nitroso compounds [52,53].

6. Fur has Long-term Effects on Gastric Tissue Cells and Creates a More Viable Environment

Ferric restriction is an intraorganismal innate immune modality of defense against infection. The epithelial barrier plays a decisive role in the isolation of bacteria that fixate on the mucosal surface. The organism effectively uses interstitial and intracellular iron due to the presence of the epithelial barrier. In vivo, iron is isolated by high-affinity chelators such as transferrin, hemoglobin, and ferritin [54]. In the inflamed gastric mucosa, neutrophils secrete lactoferrin, which binds free iron tightly on the mucosal surface, thus, effectively prevents bacterial growth using iron-dependent mechanisms [55]. Simultaneously, inflammation induces the upregulation of hepcidin, a central regulator of iron metabolism, produced by cells in the gastric gland wall. Upregulated hepcidin effectively blocks iron uptake in the small intestine, ultimately reducing pathogenic iron [54]. Infection of the organism with H. pylori triggers chronic gastric inflammation. It disrupts the hydrochloric acid-secreting glands in the stomach, ultimately leading to precancerous lesions of atrophic gastritis and intestinal chemosis [56-58]. In the gastric lumen, the mucus layer has a pH of approximately 2. 0 or higher. However, H. pylori has a short residence time in the gastric lumen before entering the mucus layer. The habitat of H. pylori has a pH range of 4. 5–6. 5; thus, the bacterium has a

high capacity to neutralize the acidity of the gastric lumen [59]. The virulence of H. pylori, host genetics, and environmental factors all contribute to the development of gastric cancer [60]. H. pylori can extract nutrients from or across polarized epithelial cells [61]. It not only uses isolated internal host iron reserves through the epithelial barrier but also directly uses dietary iron.

7. Occurrence of IDA is Closely Associated with Fur

Increasing evidence has demonstrated that H. pylori infection is associated with IDA [62]. Prior research indicates that eradication of H. pylori results in significant efficacy and effective control of refractory IDA [63]. Enhanced Fe-ion uptake in H. pylori strains isolated from individuals with IDA [64]and studies in children with IDA also suggests that H. pylori may have a strong association with the development of iron deficiency symptoms [65]. Studies in resource-poor areas have shown that the number of people with IDA approaches 30% and that H. pylori may have an important association with the occurrence of IDA in childhood [66]. Oral iron administration in IDA patients with H. pylori infection is not therapeutic, and eradication of H. pylori can restore iron homeostasis in the body [67]. In rodents and humans, H. pylori may lead to a hypoferric state, exacerbating the outcome of H. pylori infection and further induces gastric cancer [68]. Considerable evidence supports the association of unexplained iron deficiency, idiopathic thrombocytopenic purpura (ITP), and anemia with H. pylori infection [69,70]. The possible production of Fur by H. pylori interferes with dietary iron competition, decreases ascorbic acid concentration in gastric juice, affects dietary iron absorption, and upregulates pro-inflammatory cytokines and iron-regulated hormones causing IDA. Moreover, sufficient evidence suggests that Fur and the molecules involved in its regulation may interfere with the aforementioned processes [71-73].

8. H. Pylori Conventional Drug Regimens have a Novel Inhibitory Effect on Fur

Treatment targeting Fur appears to eliminate H. pylori colonization. Currently, globally accepted treatment regimens are centered on drugs containing antisecretory agents. Most commonly, they are combinations of triple or quadruple therapy drugs containing amoxicillin, clarithromycin, metronidazole, fluoroquinolones, rifabutin, bismuth, or tetracycline [74]. Attempts to overcome resistance have led to the introduction of four drug regimens that contain PPI, amoxicillin, clarithromycin, and metronidazole, called sequential, concomitant, mixed, and reverse mixed therapies [75]. With the general perception of H. pylori treatment, new studies emerge with new ideas. Traditionally, metronidazole is an antibiotic, but recent studies indicate that metronidazole may have some effect on Fur [35]. This effect increases the importance of metronidazole as a therapeutic agent for H. pylori. Furthermore, the understanding of bismuth has also changed. Bismuth was often used to inhibit gastric acid secretion and protect the gastric mucosa. However, with the intensive study of Fur, especially after the three metal binding sites of Fur were understood in depth, a new role of bismuth in the treatment of H. pylori is revealed through a simple metal substitution, thus acting as an inhibitor of H. pylori colonization [8,76,77].

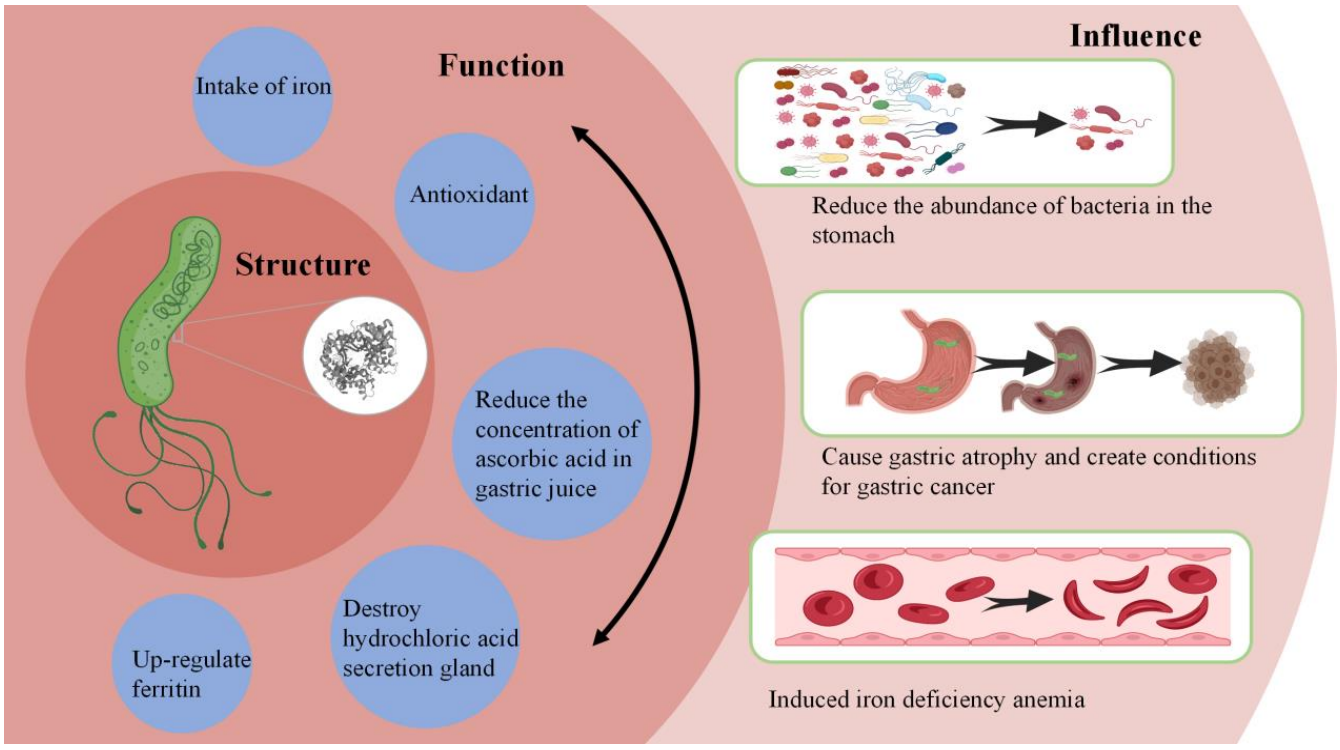

Figure 1: Fur body model (structure source: Helicobacter pylori (train ATCC 700392/26695) (Campylobacter pylori))

9. Intervention in Intragastric Iron Homeostasis May Induce Iron Death Through Environmental Differences

Several studies have shown that the administration of iron supplements significantly improves the iron content in organisms [78-80]. A clinical observation trial on drinking water with different iron levels showed that the prevalence of anemia and iron deficiency in pregnant women living in areas with high and low iron content in groundwater differed [81]. Studies on adolescents in major iron deficiency stages have shown that dietary iron intake is effective in reducing the prevalence of iron deficiency diseases [82]. In terms of the global economy, there seems to be a positive feedback aspect moderating H. pylori and low-income countries [83]. In animal experiments on dietary composition and H. pylori colonization-associated diseases, it was verified that dietary composition has a clear effect on the progression of the disease after H. pylori colonization [84]. And it was also verified in animal experiments with high salt diet, regarding the apparent involvement of Fur and its mutations in H. pylori colonization after altered intragastric environment [85]. Based on the properties of Fur and the specific environment in the stomach, it seems that a new and plausible therapeutic hypothesis for H. pylori has been considered from the point of view of interfering with iron homeostasis in the stomach. By interfering with the expression process of Fur by regulating the content of various ingested trace elements, H. pylori appears to ingest large amounts of iron, which causes oxidative stress in H. pylori and eventually leads to iron death in H. pylori. The presence of an acidic environment in the stomach can contribute to a biased ionic transformation of metal elements.

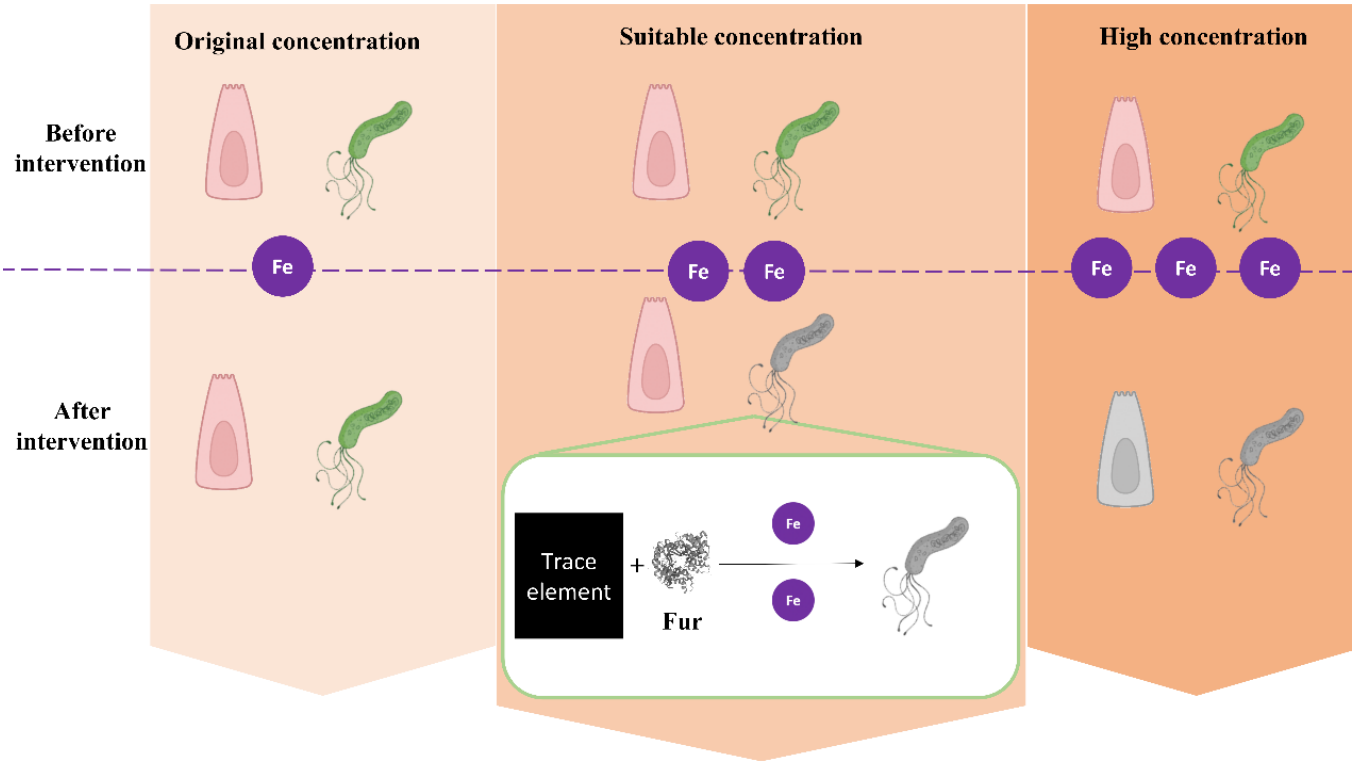

Figure 2: Fur body model

10. Reconstitution of Iron Homeostasis in the Stomach May have a Facilitative Effect on Digestive Disorders

The low pH of the stomach and proximal small intestine helps to maintain iron in a soluble form, thus making it available for absorption. Small organic acids such as citric acid and ascorbic acid also help to maintain non-heme iron in reduced and soluble forms and can greatly enhance its absorption [86]. Cellular iron homeostasis is tightly regulated to maximize iron supply in the presence of cellular iron deficiency and to limit iron supply and promote storage when cellular iron is adequate. Several studies have shown that iron fortification can alter the microbial profile in the gut, thereby promoting the growth of potentially pathogenic Enterobacter species, [87-89]. In contrast, inflammation, which is an acute phase response to infection, injury or environmental insult, can directly affect the concentration of most iron indicators [90]. H. pylori by virtue of Fur alters the intragastric environment and seizes dietary iron, leading to the development of atrophic gastritis and eventual progression to gastric cancer [91]. The mechanism of iron deficiency anemia, a complication of atrophic gastritis, is more closely associated with Fur. Rebuilding iron homeostasis in the stomach is not only a new therapeutic idea for targeting H. pylori, but also has a positive guiding effect on dietary iron intake and absorption.

11. Discussion

H. pylori is one of the most important contributors to the pathogenicity of the digestive system, and studies have confirmed that its infection can lead to peptic ulcers, atrophic gastritis, and gastric cancer [92,93]. The resolution of H. pylori by microbiologists, including its structure, pathogenesis, escape mechanism, and metastasis, has been more comprehensively understood with time [94,95]. When we systematically analyzed the studies related to H. pylori,

Fur seems to exhibit unique importance at several levels of H. pylori. It is to be noted that Fur is not unique to H. pylori. Most known bacteria contain Fur, which regulates iron uptake, and even these bacteria have a more comprehensive protein uptake of the metallic ion [96]. When evaluated from the perspective of metal uptake, H. pylori is even inferior to other bacteria [97,98]. However, the wisdom of microorganisms should not be underestimated. Microorganisms are more ancient species than humans, and their survival strategy is worth learning in terms of environmental adaption. H. pylori was the first bacterium that colonized the stomach and induced various pathological changes in gastric tissues. Hence, the uptake and escape mechanisms of its survival are especially worth studying, and the elements involved in these mechanisms have a close relationship to Fur. The digestive system hosts a variety of living organisms, and the study of intestinal flora has become an important field of research. The intestinal flora influences the occurrence of several diseases, which are not only digestive but also cardiovascular in nature. The study of microorganisms in the digestive system cannot be limited to the intestinal tract, as traces of microorganisms have been found in the oral cavity, esophagus, and stomach. The new study overturns the traditional knowledge that bacteria cannot colonize the stomach, and the number of bacteria detected in the stomach does not differ significantly from that detected in other parts of the digestive system. The Fur of H. pylori and the proteins involved in the regulation of Fur control the uptake of various metal elements by H. pylori and can effectively resist oxidative stress and complete the colonization of H. pylori. In addition, after H. pylori colonization in the stomach, Fur will continue stimulating hydrochloric acid-secreting glands in the stomach and inhibiting gastric acid secretion. Thus, it creates a microenvironment more suitable for the survival of H. pylori, and such stimulation may lead to atrophic gastritis and intestinal chemosis. Some patients also develop IDA to varying degrees after the onset of atrophic gastritis, and further evidence suggests that Fur is involved in developing IDA. Moreover, the use of anti-H. pylori drugs in the treatment of IDA are more effective than the use of iron supplements alone [99,100]. In the conventional treatment regimen for H. pylori, drugs such as bismuth and metronidazole seem to have effects on Fur, also to varying degrees. This might account for the positive effect of these drugs in H. pylori treatment. The significance of Fur makes it impossible to ignore its existence in the study of H. pylori. Fur has an important role in the colonization of H. pylori, and Fur appears to be influenced by a variety of trace elements, including iron. By regulating a variety of trace elements, which further regulate Fur and ultimately affect the colonization of H. pylori, altering iron homeostasis appears to be an effective means of inhibiting Fur as well. In addition to the factor that H. pylori and the host belong to two species, the strong acidic environment in the stomach also acts as a catalyst for chemical reactions that create favorable factors for the altered iron homeostasis environment. The study of Fur may become a new research direction and play a significant role in treating H. pylori. Fur is related to the colonization of H. pylori and has an important impact on the progression of many digestive diseases. Studies on ferroptosis opened new cell death pathways under metal-ion induction, and further copper death also confirms the importance of this new pathway. The study of iron death is not only relevant to cancer cells, but iron uptake is a necessary task for most living organisms. Notably, the use of metal element concentration differences to target the treatment of different individuals concerning the microenvironment may become a new direction in studying iron death. Our present study provides a reliable basis to advance this research further.

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