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Toll-Like Receptors as Biomarkers of Gastric Carcinogenesis: Implications for Diagnosis, Prognosis and Treatment

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ABSTRACT

Toll-like receptors (TLR) are essential for *Helicobacter pylori* (Hp) recognition and subsequent innate and adaptive immunity responses. TLR2 appears to be the receptor responsible for most of the immunologic reaction against Hp infection. However, TLR4, TLR9 and eventually TLR5 may also have a synergic effect with TLR2 against Hp. It has been shown that gastric Hp infection increases TLR expression in the gastric mucosa. Moreover, recent studies have shown that human gastric carcinogenesis is associated not only with increased expression of TLR but also with decreased expression of their inhibitors such as Toll-Interacting Protein (TOLLIP) and peroxisome proliferator-activated receptor (PPAR)-γ. Indeed, gastric dysplasia and adenocarcinoma are associated with high expression levels of TLR and low levels of TOLLIP and PPAR-γ, suggesting increased activation of these receptors throughout human gastric carcinogenesis. In this article we discuss how these novels findings could be used not only for the diagnosis and prognosis of gastric lesions associated with Hp infection but also for their treatment. Specifically, we discuss the potential use of TLR agonists in addition to antibiotics to improve eradication rates of Hp and of TLR antagonists to slow the progression of gastric preneoplastic lesions. We also discuss the potential value of TLR signalling blockers and quantification of tumoral TLR expression, respectively, in the treatment and prognosis of gastric cancer. In conclusion, TLRs can be an important link between Hp and the sequence of gastric carcinogenesis and they can be used as biomarkers of gastric carcinogenesis. In this article, future lines of investigation related with these novel scientific findings are proposed and discussed.

Keywords: Toll-Like Receptors; Gastric Cancer; TOLLIP; Carcinogenesis; *Helicobacter pylori*

1. Introduction

Gastric pathology has some unique characteristics mainly because many gastric diseases have a strong association with a bacteria infection—*Helicobacter pylori* (Hp). Indeed, this bacteria discovered in the 1980s was rapidly associated to several gastric pathologies [1]. In 1994 it was clearly recognized that Hp was a major cause of gastroduodenal peptic ulcers and later that year the International Agency for Research on Cancer declared Hp to be a group I human carcinogen for gastric adenocarcinoma [2]. Hp is considered one of the oldest bacteria to infect humans with genetic studies identifying this bacterium in the first human populations, more than 58,000 years ago [1]. Even today with wide use of antibiotics, Hp is estimated to infect more than 50% of the World's population, with prevalence's as high as 90% in some developing countries [1].

Hp is a Gram-negative bacterium that adheres to the surface of gastric mucosa, without invasion of gastric epithelial cells, and that upon interaction with several innate immunity receptors such as Toll-like receptors (TLRs), causes inflammation of the mucosa that perpetuates as a chronic gastric inflammatory state [3,4]. In some patients this inflammation progresses leading to gastric atrophy and intestinal metaplasia, clearly established gastric premalignant gastric conditions [5-8]. Indeed, Correa was the first one to describe a multistep pathway for the intestinal-type gastric adenocarcinoma, where Hp is considered the initiator of the so-called

Correa cascade of gastric carcinogenesis that involves chronic gastritis, atrophic gastritis, intestinal metaplasia, gastric dysplasia and finally, intestinal-type gastric adenocarcinoma [9-12]. The innate immune system is the first line of defense against several microbial agents, consisting of a diversity of components that initiate protective immunological responses [13,14]. Although many of these factors can prevent or destroy the invading pathogens non-specifically, we now know that the microbiological recognition by innate immunity is also a specific and highly coordinated process involving pattern recognition receptors (PRRs) that identify preserved structures of different pathogens, the so-called pathogen-associated molecular patterns (PAMPs) [13,14]. Moreover, this amazing specificity conferred by the recognition of PAMPs by PRRs is essential, not only for a more adequate initial control of a potential infection (innate immunity), but also for triggering the late antigen-specific acquired immunity (adaptive immunity), for controlling inflammation processes and for maintenance of a immunological homeostasis within the host [15,16].

The toll-like receptors (TLRs) are the most important class of pathogen-associated molecular patterns (PAMPs) receptors, with ten different TLRs being ubiquitously expressed in humans [14,17-19]. TLRs are surface molecules on eukaryotic cells, present in invertebrates and conserved in vertebrates, which were originally identified as homologs of Drosophila Toll molecule, an important component of antifungal defence mechanism [14,20,21]. The existence of several TLRs enables the innate immunity system to recognize different groups of pathogens while initiating appropriate and distinct immunological responses, according to the PAMP recognized [13,17, 22]. In normal physiological conditions TLRs do not recognize self-ligands. However, after tissue lesion they may recognize endogenous antigens, the so-called damage-associated molecular patterns (DAMPs), and contribute to promote sterile inflammation [23,24]. Although initially described in several immunological cells, various studies have shown that different human tissues express these receptors, with the degree of expression varying from tissue to tissue [14,17,18,20,21].

The structure of all TLRs is identical. TLRs are membrane-surface receptors consisting of a distinct leucine-rich repeat (LRR) extracellular domain that confers specificity to the receptor, a single transmembrane domain and a conserved toll/interleukin 1 (IL1) receptor (TIR) intracellular domain, homologous to the IL1 receptor [17,18]. In general, TLR2 recognizes PAMPs mainly from Gram positive bacteria, TLR4 is the recaptor for Gram negative bacteria lipopolysacharide (LPS), TLR5 recognizes bacteria flagellin, TLR3, TLR7 and TLR8 recognize viral components namely double (TLR3) and single-stranded RNA (TLR7/8), TLR9 recognizes unmethylated CpG DNA from bacteria and virus, and finally, TLR1 and TLR6 form heterodimers with TLR2 in order to sense tri-acyl (mycobacterium) and di-acyl lipopeptides (mycoplasma), respectively. In addition, TLR4 and TLR2 can detect a wide range of antigens not only from bacteria but also from fungus, parasites, virus (particularly TLR2) and DAMPs (particularly TLR4) [14,25].

The coupling of TLRs with its respective ligand initiates intracellular signalling pathways leading to the production of several inflammatory cytokines such as TNF-α, IL-1 and several others inflammatory molecules through the activation of nuclear factor-κB (NF-κB) [17,26-29]. Despite similar intracellular signalling pathways, the final result of stimulating different TLRs is not exactly the same depending not only of the activated receptor but also of the cell that is stimulated [30-32]. Moreover, because they are intrinsically related to inflammation but also to cell survival signalling, epithelial regeneration and cell proliferation, recent reports associate these receptors function to tumorigenesis [33,34]. Indeed, it appears that the perpetuation of TLRs signaling pathways, such as in Hp-induced chronic gastritis, confers oncogenic potential to the cells [17,26-29,33,34]. In that way, in the gastrointestinal tract, in order to prevent inadequate inflammatory responses to non-pathogenic antigens, a strict regulation of these receptors activity is fundamental for maintaining homeostasis [4,20,35-46]. For that reason, normal gastrointestinal mucosa expresses low levels of TLRs and high levels of several TLR-antagonists, like Toll-interacting protein (TOLLIP) and PPAR-γ, molecules that block TLR signalling pathways and NF-κB activation, respectively [4,20,35-46]. This further underscores a complex and not completely understood intracellular signalization for these receptors.

Recent studies suggest that TLRs not only have an important role in Hp recognition but also in the progression of gastric lesions associated to this infection. In this article we will discuss the fundamental role of TLRs in the immunological response to HP infection and in the progression of gastric preneoplastic lesions and how this novel finding could potentially be used in clinical practice.

2. *Helicobacter pylori* Immune Recognition by Toll-Like Receptors

Several studies demonstrate that TLRs have an essential role in Hp recognition and subsequent innate and adaptive immunity against this bacterium [47-61]. After the first contact with the gastric mucosa Hp appears to interact with several TLRs inducing an immunological and inflammatory response [47]. Moreover, being Hp a bacterium that causes marked inflammation without invasion

of gastric epithelial cells it looks clear that this first line of interaction is crucial for the subsequential cascade of inflammation induced by this bacteria [3]. However, which is the principal TLR responsible for this process is a question of some controversy.

Different TLRs may play a role in gastric immunologic response to Hp [4]. Although Hp is a Gram-negative bacteria that possesses LPS, the main ligand of TLR4, TLR4 does not appear to be the principal TLR responsible for the immunologic response to Hp [3]. Actually, TLR2, a TLR that recognizes several antigens from Gram-positive bacteria, appears to be the receptor responsible for most of the inflammatory changes occurring as the result of Hp infection [4,47,49].

Indeed, some studies showed that TLR2, but not TLR4, was required for Hp-induced NF-kappa B activation and cytokine production both by epithelial [56] and antigen presenting cells [57]. Moreover, cytotoxin-associated gene A (Cag A), an important virulence factor of Hp, promotes a higher production of inflammatory cytokines by TLR2 and not TLR4 signalling [58]. On the other hand, some studies also suggest that TLR4 recognizes several Hp antigens and, in that way, also plays an important role in Hp infection [59-61]. In fact, more conclusive studies demonstrate that either in epithelial or dendritic cells, TLR2 appears to be the principal receptor for recognition of Hp. However, this process depends also in some extent of TLR4 that acts in synergy with TLR2 [47-52]. The data regarding the role of TLR5 (an extracellular receptor for bacteria flagellin) on Hp recognition are highly controversial. Although Hp is a flagellated bacteria, it appears that the flagellin of ε-Proteobacteria, including Hp, is barely recognized by TLR5 [59-61]. Some initial studies suggested an interaction between Hp flagellin and TLR5 leading to production of several pro-inflammatory cytokines [49,62]. This was refuted by other studies demonstrating that TLR5 was unresponsive to Hp flagellin [59-61]. These studies suggested that non-recognition of Hp flagellin by TLR5 could be a mechanism for Hp immune evasion [59-61]. More recent studies suggested that TLR5 was important for Hp recognition and inflammatory response to these bacteria, adding more controversy about this subject [63]. Taking altogether, concerning TLR5, the data are contradictory and TLR5 may have none or little role in Hp recognition.

Concerning the intracellular TLRs, TLR9 is the only one that recognizes Hp DNA and appears to have a complementary and synergistic action with the other two receptors (TLR2 and TLR4) [49,64].

So, in conclusion, TLR2 by recognizing several Hp antigens appears to be the receptor responsible for most of the immunological process that occurs as the result of Hp infection. However, TLR4 and TLR9 recognize other Hp antigens and have a synergistic role with TLR2. The contradictory data regarding recognition of Hp flagellin by TLR5 suggest that this TLR has no or small role in Hp induced immune response.

3. Toll-Like Receptors and the Progression of Gastric Preneoplastic and Neoplastic Lesions

Although data associating TLRs with the progression of gastric lesions are scarcer than data concerning Hp-recognition by these receptors, recent studies suggest that TLRs may also have an important role in gastric carcinogenesis.

Initial studies showed that chronic Hp infection increased TLR4 expression and NF-κB activation [60,61]. Later on, using immunohistochemistry, our group and others showed that intestinal metaplasia, dysplasia and carcinoma cells expressed TLR2, TLR4, TLR5 and TLR9 in a more intense and diffuse way when comparing with normal mucosa [65,66]. Moreover, our group confirmed also at a genetic level this increased TLR expression through the progression of gastric carcinogenesis, particularly TLR2 and TLR4 [67]. More importantly, we demonstrated that not only TLR RNA and protein levels were increased but also TOLLIP and PPAR-y levels, important TLR antagonists, were significantly decreased through the progression of gastric preneoplastic and neoplastic lesions [67].

In fact, we demonstrated that epithelial cells from normal mucosa colonized by Hp (gastritis) express two times more TLR2 and 4 (but not TLR5) when compared with normal mucosa without Hp [67]. Furthermore, the expression of TLRs antagonists TOLLIP and PPAR-y in gastritis was decreased in 25% when compared with normal Hp negative mucosa [67]. This is very important since this decrease of TLRs antagonists not only suggests increase activation of TLRs pathways but it can directly interfere with TLRs protein levels. Actually, as we have shown in colon mucosa, a decrease in TOLLIP levels associates with higher TLR protein expression even with similar levels of RNA [68]. Indeed, besides antagonizing many intracellular kinases activated by TLRs, TOLLIP also blocks TLRs molecules and promotes traffic of newly synthesized proteins into endosomes leading to TLR early degradation, meaning that lower levels of TOLLIP are associated with more functional TLRs [69-74].

Gastric intestinal metaplasia presents even lower PPAR-γ and TOLLIP expression, with 30% to 40% less expression when comparing to normal mucosa, and 1.2 - 1.5 times more TLR2 and TLR4 expression now dispersed trough the entire cell [67]. In dysplasia and cancer the expression of TLR RNA is similar to intestinal

metaplasia. However, PPAR-γ and TOLLIP levels are even lower, almost half of the expression when compared to normal mucosa, and this translates in a more intense and diffuse TLR protein expression throughout all the cell [67]. In fact, this significantly decreased TOLLIP expression altered the distribution of TLR protein expression that was polarized at the cellular membrane (particularly basolateral membrane) in normal epithelial cells and diffuse trough the cytoplasm and membrane in intestinal metaplasia, dysplasia and cancer [65-67]. The effect of Hp on the genetic profile of the mucosa is higher in normal mucosa than in intestinal metaplasia since in normal mucosa Hp highly augments the expression of TLR2, TLR4, TNF-α and decreases TOLLIP and PPAR-y and in intestinal metaplasia it only augments in a low level TLR4 and TNF-α without a significantly change in the expression of TLRs antagonists. In dysplasia or cancer the presence of this bacterium does not appear to change the genetic profile of the mucosa [67].

In our opinion it is the progressive activation of the overexpressed TLRs induced by Hp that will eventually lead to aberrant transcription of CDX2 and phenotypic change to intestinal metaplasia (**Figure 1**), similarly to what was previously shown in biliary epithelium [75]. Intestinal metaplasia with higher TLRs and lower expression of its antagonists is probably more reactive to several PAMPs, including from other microorganisms. However, at this stage the presence of Hp can remain of some importance since the mucosa is still reactive to this bacterium that, as we have shown, doubles TNF-α levels in the mucosa and also increases TLR4 and CDX2 expression [67]. This increase in CDX2 induced by HP in intestinal metaplasia cells can be of some importance since CDX2 has been associated to progression of gastric lesions and cancer [76,77].

Progressive activation of TLRs in intestinal metaplasia will eventually lead to dysplasia and cancer, characterized by even lower TOLLIP expression and consequently diffuse TLR expression throughout the entire cell. At this stage, we believe that this intense expression of TLRs make the cell more reactive to several PAMPs from different microorganisms, even commensal ones, and even DAMPs and, so, the effect of Hp becomes negligible (**Figure 1**).

Although it is now clear that the progression of gastric lesions is associated with increase expression of TLRs and lower expression of its antagonists, which suggests overactivation of TLRs signalling pathways, we do not know for sure if these receptors are functional and leading to the production pro-inflammatory and pro-oncogenic mediators at these later stages of gastric carcinogenesis. However, as gastric metaplastic, dysplastic and carcinoma cells express several TLRs, an interaction with Hp and with other microorganisms is probable [66]. Indeed, in cell lines studies it was shown that several PAMPs via interaction with epithelial TLRs can induce gastric carcinoma-promoting factors, such as IL-1, IL-8 and angiogenic factors [56,66,78]. Moreover, Hp appears to augment the growth of gastric cancer cells through the LPS-TLR4 pathway, promoting proliferation and progression of gastric cancers [79]. Other studies showed that Hp activation of NF-κB through TLR2 and TLR9 was able to promote cyclooxygenase-2 (COX-2) overexpression, invasiveness and angiogenesis of gastric cells [27,80]. Other suggested that PAMPs from other microorganisms may also promote tumor growth via TLR2

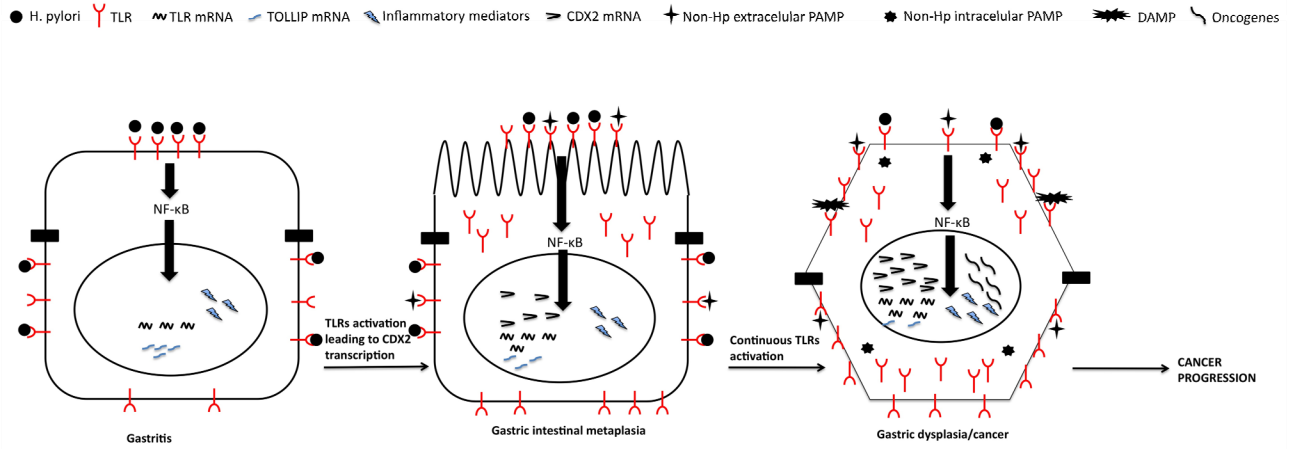

Figure 1. TLRs progressive activation in gastric carcinogenesis. Sequence from normal mucosa to gastric cancer involving progressive TLRs activation, initially only to *Helicobacter pylori* but later on eventually to several different PAMPs and even DAMPs. *Helicobacter pylori*-dependent TLRs chronic activation throughout the years leads to chronic inflammation that eventually leads to aberrant transcription of CDX2 with phenotipical change to intestinal metaplasia. At this point other agents may promote chronic inflammation with production of oncogenic mediators that eventually will lead to dysplasia/ cancer.

signaling, independently of Hp [81]. So, these in vitro studies gave indirect evidence that the overexpressed TLRs that we saw in human gastric biopsies are in fact functional and may play a crucial role in gastric carcinogenesis.

In some way confirming this very important role of TLRs in gastric carcinogenesis, some studies described an association of TLR4, TLR2 and even TLR5 polymorphisms with the risk of developing gastric preneoplastic lesions and gastric cancer [82-86].

In conclusion, although more data is needed in this area, several in vitro, translational and polymorphisms human studies suggest that TLRs may have a crucial role in gastric carcinogenesis.

4. Potential Diagnostic and Prognostic Value of Toll-Like Receptors in Gastric Lesions

Scarce scientific evidence exists concerning the clinical application of the previous described data on the role of TLRs for Hp recognition and progression of gastric lesions. However, we believe that in the future this knowledge can acquire an important role not only for the diagnosis of gastric preneoplastic lesions but particularly for the prognostic of gastric neoplasias.

Concerning the diagnosis of gastric lesions, we have shown that TLRs immunohistochemistry quantification could be used for the differentiation of the different gastric lesions [65]. When adding the relative expression of TLR2, 4 and 5, we obtained several expression scores (from 0 to 9), with different scores associating to different lesions. For instance, the presence of an expression score of 1 seems to leads to a very low false negative rate (<1%) for lesions as severe as dysplasia. On the other hand, to adequately identify dysplasia a score of 8 seems to be very useful as it leads to a very low false positive rate (4%) in patients with precancerous conditions and also to a low false positive rate (missing invasiveness of 9%) when distinguishing dysplasia from invasive cancer [65]. In practical terms we may say that we could use the fact that gastric dysplastic cells have a strong expression of TLRs for an accurate diagnosis of gastric dysplasia. Indeed, sometimes when there are marked inflammatory and/or regenerative epithelia changes it is difficult for the pathologist to differentiate between these benign alterations from dysplasia. In this particular case we believe that TLRs immunohistochemistry quantification may help in the differentiation of inflammation (low to moderate expression) with dysplasia (very high TLRs expression).

Moreover, we may speculate that this different TLR expression in gastric lesions may also help the endoscopic diagnostic of lesions. Current guidelines still recommend histology as the gold standard, however, it was recognized that new endoscopy technologies might help the diagnosis of gastric lesions [87]. In fact, our group demonstrated that narrow-band imaging (NBI) was highly accurate for the diagnosis of gastric preneoplastic and neoplastic lesions [88]. However, the problem of these new techniques is that they can be cumbersome and, until now, they rely on previous mucosal changes identified on standard white-light endoscopy, and only then they can be applied. We may speculate that antibodies-marked TLRs that release, for example, fluorescence waves upon interaction with white light would help the diagnosis of lesions, by creating fluorescence hot-spots for dysplasia that could easily be recognized in a standard endoscopy. We believe that future studies should have this in mind.

As for predicting gastric cancer risk or prognosis, all that can be said is only speculative, since to our knowledge no single study analyzed if TLRs epithelial or tumoural expression has prognostic/predicting value or not. Nevertheless, as we have discussed it appears that TLRs are active in tumoural cells producing several inflammatory, survival, angiogenic and oncogenic mediators that may facilitate tumorogenesis and cancer progression. So, in theory, TLR expression in tumours may in fact have prognostic value and may act as biomarkers, with worse prognosis seen in tumours with higher TLR expression. Indeed, in colorectal cancer it has been shown that higher TLR4 expression confers worse prognosis [89]. Moreover, several TLRs polymorphisms, including from TLR2 and 4, were associated with colorectal cancer development and prognosis [90]. In oesophageal adenocarcinoma increased TLR9 expression was also associated with adverse prognosis [91]. If TLR expression can influence prognosis of other gastrointestinal tumours, we believe that it can alter also the prognosis of gastric cancer. Future studies are needed to evaluate this aspect.

5. Potential Therapeutic Value of the Modulation of Toll-Like Receptors

Taking together the important role of TLRs for Hp recognition and immune response as well as the potential role in the progression of gastric lesion we may preview several lines for therapy (**Table 1**). The first one is vaccination—can TLR modulation help in the creation of an efficacious vaccine against Hp infection? The true is that until now no vaccine, prophylactic or therapeutic, using whole inactivated Hp, Hp proteins or recombinant vaccines, has shown to be efficacious against this infection in humans [92,93]. In theory, TLRs agonists as adjuvant to the vaccine can improve efficacy rates. However, until now, to our knowledge no study has evaluated this aspect.

Other line of potential research is using TLRs agonists

Table 1. Potential therapeutic targets for Hp-TLRs interaction in clinical practice.

Clinical scenario	Scientifical evidence	Modulator of TLR	Therapeutic target
Hp vaccine	TLR2, 4 and 9 promote production of several inflammatory mediators when in contact with Hp	TLR2, 4 and 9 agonists	Enhanced immune response to Hp vaccine
Hp eradication		TLR2, 4 and 9 agonists	Higher Hp eradication rates with antibiotics
Gastric cancer prevention	Gastric carcinogenesis is associated with increased levels of TLRs and decreased TLRs-inhibitors like TOLLIP	TLR2 and 4 antagonists TOLLIP stimulators	Decreased inflammatory environment with slowed rate of gastric pre-malignant lesions progression
Gastric cancer treatment	Gastric cancer express high levels of TLRs Immune cells may fight cancer cells	Topic TLR antagonists directed to cancer cells Systemic TLR agonists directed to immunological cells	Double action against cancer—direct inhibitory action on cancer cells and enhanced immune anticancer therapy

as adjuvant not to a vaccine but to antibiotherapy for treatment of Hp established infection. Indeed, current eradication rates with standard multidrug regimens are not ideal. We may speculate that using TLRs agonists with antibiotics may increase eradication rates and may help to overcome antibiotic resistance of these bacteria. However, once more, until know no study has evaluated this aspect. We believe that both for vaccination and to therapy future studies should evaluate the role of TLRs agonists. However, we should keep in mind that the effect of these substances is not linear since some studies show that stimulation of these receptors may in some circumstances have an anti-inflammatory effect. Indeed, although TLR2 is considered the most important receptor for HP recognition and subsequent immune response it appears that under some circumstances TLR2 can induce production of anti-inflammatory cytokines and, in this way, inducing immunotolerance [49]. Furthermore, TLR9, another important recognition receptor for HP may have an anti-inflammatory effect on the early phase of gastritis [94]. So, although TLR stimulation may have a role in Hp vaccine and eradication, these aspects should be kept in mind in future studies.

Another potential line of treatment, and eventually even more important one, is the role of TLR modulation for prevention of gastric cancer in patients with premalignant lesions. As we have seen progression of gastric lesions is associated with increasing levels of TLRs and diminished TLRs inhibitors. The question is: If we block TLRs or if we are able to increase its inhibitors (TOLLIP) can we prevent cancer development? No study has investigated this question, however, in theory, if we block TLRs we will decrease its stimulation and consequently diminish production of pro-inflammatory and pro-oncogenic mediators. Although a direct antagonism of TLRs can be feasible, particularly if these substances act only topically in the gastrointestinal tract, a substance that can modulate TLRs activation by augmenting TOLLIP levels, for example with probiotics, would be even more attractive since the risk of infection would be theoretically lower with these substances. This could be a very important line of research since at this moment, besides surveillance, we have nothing more to offer to these patients.

Finally, since TLR stimulation on malignant cells leads to production of cancer promoting factors, we must consider that TLR antagonists may a role in gastric cancer treatment. Interestingly, TLR agonists and not antagonists have been used for cancer treatment as a form of immunotherapy. However, if TLR agonists may promote an immune reaction against cancer, the true is that a direct effect on tumours may have an opposite effect. So, in our opinion a strategy of a topical action of TLR antagonism directly on cancer cells with a systemic TLR activation of immune cells could promote a double attack against cancer. This is a strategy that deserves further study since gastric cancer is one of the most lethal cancers in the world.

6. Conclusions

As we have seen TLRs have a crucial role in Hp recognition and subsequent immune response against this bacteria. Furthermore, they may have an essential role in the progression of gastric lesions associated with Hp leading to cancer since the progression of gastric lesions is associated with increasing levels of TLRs. Although the role of TLRs in the physiopathology of gastric lesions is established, scarce humans studies exist concerning the potential use of Hp-TLRs interaction in clinical practice. In this article we suggested that TLRs may act as biomarkers of gastric carcinogenesis and several lines for future research considering the important role of TLRs in gastric disease were presented. Namely, we suggested that these receptors should be evaluated in the prognosis of gastric cancer. Moreover, we provide several lines for translational and human research using TLRs modulation not only for vaccination and HP eradication but also for the prevention of the progression of gastric preneoplastic lesions and also to the treatment of gastric cancer.

In conclusion, TLRs can be the link between Hp and the sequence of gastric carcinogenesis. Future lines of

investigation should study these novel and enthusiastic scientific findings in order to obtain more weapons in the fight against gastric cancer.

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