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Helicobacter pylori, gastric microbiota and gastric cancer relationship: Unrolling the tangle

Liatsos C et al. *Hp*, gastric microbiota and gastric cancer

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Abstract

Helicobacter pylori infection (*Hp*-I) represents a typical microbial agent intervening in the complex mechanisms of gastric homeostasis by disturbing the balance between the host gastric microbiota and mucosa-related factors, leading to inflammatory changes, dysbiosis and eventually gastric cancer. The normal gastric microbiota shows diversity, with *Proteobacteria* (*Hp* belongs to this family), *Firmicutes*, *Actinobacteria*, *Bacteroides* and *Fusobacteria* being the most abundant phyla. Most studies indicate that *Hp* has inhibitory effects on the colonization of other bacteria, harbouring a lower diversity of them in the stomach. When comparing the healthy with the diseased stomach, there is a change in the composition of the gastric microbiome with increasing abundance of *Hp* (where present) in the gastritis stage, while as the gastric carcinogenesis cascade progresses to gastric cancer, the oral and intestinal-type pathogenic microbial strains predominate. *Hp* infection creates a premalignant environment of atrophy and intestinal metaplasia and the subsequent alteration in gastric microbiota seems to play a crucial role in gastric tumorigenesis itself. Successful *Hp* eradication is suggested to restore gastric microbiota, at least in primary stages. It is more than clear that *Hp* infection, gastric microbiota and

gastric cancer constitute a challenging tangle and the strong interaction between them makes it difficult to unroll. Future studies are considered of crucial importance to test the complex interaction on the modulation of the gastric microbiota by *Hp* as well as on the relationships between the gastric microbiota and gastric carcinogenesis.

Core Tip: Gastric adenocarcinoma is a leading cause of cancer-related death in the world. Chronic gastric infection caused by *Helicobacter pylori (Hp)* is the strongest identified risk factor for gastric adenocarcinoma, prompting the World Health Organization to classify it as a class I carcinogen. It has been shown that, in *Hp*-colonized patients, this pathogen accounts for more than 90% of all gastric microbiota modifying healthy microbiota and reducing its overall diversity. In this review, we tackle the complicated relationship between *Hp*, gastric microbiota and gastric cancer in an effort to unroll this tangle.

INTRODUCTION

Gastric cancer (GC) has been recognized as a global health concern; it is still the fifth most frequent global malignancy and one of the main causes of cancer-related death[1]. Likewise, *Helicobacter pylori* infection (*Hp*-I), an important public health burden affecting more than half of the global population[2], is related with the majority of GC, with an estimate between 74.7% to more than 90% of the new non-cardia GC cases[1,3].

Regarding the interaction between *Hp*-I and GC, relevant mechanisms known for many years have been studied and are constantly being enriched with new data (Figure 1)[4-17].

In this regard, arising evidence indicates, that *Hp,* as the most important member of abnormal gastric microbiota (GM), might induce gastric microbiome modifications[11], thereby possibly leading to gastric oncogenesis. The gastric flora may be involved in the *Hp*-related oncogenicity, and the variations in the GM composition of patients with GC, intestinal metaplasia (IM), and chronic gastritis are defined[18]. For instance, *Campylobacter* is among the most influential genera in *Hp*-associated atrophic gastritis and gastric atrophy−induced alterations of the GM, namely gastric dysbiosis, might contribute to gastric tumorigenic effect[1]. Moreover, *Hp*-related metabolic syndrome induces dysbiosis of gastrointestinal tract (GIT)- microbiota, thereby contributing to lower and upper GIT carcinogenesis, including GC[19-21]. However, the interaction between the host, microbiota, and *Hp* in the pathogenesis of GC still has to be fully elucidated[22].

Based on recent data, this review attempts to unroll the tangle regarding the interaction between *Hp*-I, GM and GC.

GASTRIC MICROBIOTA COMPOSITION

The GIT (mainly intestine) is colonized by 1-4 x10^{15} microorganisms, co-existing in a balanced relationship[22]; the GIT microbiota is estimated to be up to 2 kg and affects health and disease[23]. The majority of the bacteria found in the adults' gut consists of *Bacteroides, Parabacteroides*[23]. The anaerobic environment of intestinal lumen does not facilitate aerobic pathogens colonization and development under normal conditions, though anaerobic and facultative pathogenic species can invade it and promote diseases. Each site of the GIT has a unique distribution of microflora; when compared with the stomach and duodenum, bacteria density increases in the jejunum/ileum and colon. To yield the optimal conditions for their common interaction and survival, host and microbes have developed specific mechanisms; the disruption of those mechanisms triggers an imbalance in microbial species abundance, termed dysbiosis, which is incriminated for gut barrier dysfunction and induction of inflammatory response. In this regard, the failure to regulate the composition (microbial diversity), probably occurs

during the beginning and course of several diseases, including malignancies, such as GC[24].

Until recently, the gastric environment was considered as sterile, probably due to increased acidity, and the microbiota was believed to be isolated in the small intestine and colon. Subsequently, identifying *Hp* focused the attention on the gastric microbiota, as "an ecological niche for bacteria"[23]. Emerging data have revealed that there is a broad range of microorganisms in the stomach, with a density of 10^1 to 10^3 colony forming units/g[25,26]. Gastric microbiome is composed of bacteria ingested mainly through the ororespiratory tract, and secondary from the intestine by transpyloric biliary reflux[27,28]. Most of those microorganisms cannot resist to indigenous gastric defensive mechanisms, and there are data indicating which permanently colonize the gastric mucosa, other than *Hp*. Relative reports suggested that the predominant phyla in the gastric mucosa consist of *Streptococcus, Rothia, Lactobacillus, Veillonella, Prevotella, Neisseria*, and *Hemophilus*, counting more than one hundred sorts[28,18]. Specifically, *Hp*, represents the most important member of the GM family with the highest relative abundance. Additional GM includes *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes* and *Fusobacteria*, being the 5 most abundant phyla[18], in children and adults[29]. In culture-based studies, where cultures of gastric juice or mucosa biopsies were examined, numerous members of the *Firmicutes, Proteobacteria, Actinobacteria,* and *Fusobacteria* phyla were identified, while yeasts were recognized in relatively low abundance[30,31]. Laboratory molecular techniques with high sensitivity indicated that *Streptococcus, Prevotella, Neisseria, Veillonella* and *Rothia* represent the main bacterial populations in the gastric tissue, with Streptococcus being the most dominant genus[32-36]. Sung et al[37] revealed heterogeneity in the flora of gastric fluid and mucosa. Gastric mucosa has greater flora richness, while gastric juice has greater flora diversity[37]. The presence of bacteria in gastric juice could be just transient as a result of their ingestion with food, drinks, or saliva without colonizing the gastric mucosa, so they create a fictional image of the real diversity[18].

More specifically, Bik et al[36] by introducing a small subunit 16S rDNA clone library approach, described a diverse population of 128 phylotypes (totally 1833 bacterial isolates

obtained from gastric biopsies of 23 healthy adults) within gastric mucosal samples with the majority of bacteria belonging to the five abovementioned major *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes,* and *Fusobacteria* phyla[36]. A lot of similar studies confirmed the presence and proportion of these phyla[4,38-41]. Table 1 shows the taxonomy of most prevalent GM at phylum and genus level.

IMPACT OF *HP*-I ON GASTRIC MICROBIOTA COMPOSITION

Regarding *Hp*-I, its impact on the GM remains to be clarified. While Bik et al[36] did not depict an impact of the occurrence of *Hp* in gastric biopsies on the composition of GM, several subsequent studies characterize *Hp* as the regulator of the GM community. Andersson et al[42] revealed that *Hp* was the dominant bacterium whenever isolated, though its absence was associated with a diverse microbiota. Analytically, in samples from *Hp(+)* individuals, *Hp* was the mainstay specie (ninety percent) of the samples examined by four hundred fifty four pyro-sequencing. Thirty three phylotypes were recognized solely, two hundred twenty nine less when compared with *Hp*(-) individuals[42]. The abovementioned signifies that *Hp* has inhibitory effects on the colonization of other bacteria, harboring a significantly lower diversity of them in stomach. The GM in *Hp* negative patients was mainly dominated by the same phyla, though with diverse percent abundances: 52.6% *Proteobacteria,* 26.4% *Firmicutes,* 12% *Bacteroidetes* and 6.4% *Actinobacteria*[43]. The common genera observed in *Hp* negative individuals included *Gemella, Prevotella,* and *Streptococcus*[42].

In another study, by introducing DNA microarrays to characterize the GM in 12 corpus biopsy samples (eight *Hp* positive), Maldonado-Contreras et al[44] isolated 44 phyla with four dominant *Proteobacteria, Firmicutes, Actinobacteria,* and *Bacteroidetes. Hp*-I augmented the relative abundance of non- *Hp* - *Proteobacteria, Spirochaetes,* and *Acidobacteria* whereas lessening the relative abundance of *Actinobacteria, Bacteroidetes* and *Firmicutes,* compared to uninfected stomachs[44]. An additional study from Mongolia, showed that patients infected with *Hp* exhibited a significantly lesser bacterial richness and Shannon and Simpson indices[45,46] compared with *Hp* negative arms. Moreover, enrichment of

Firmicutes, Fusobacteria, Bacteroidetes and *Actinobacteria* at phylum level was shown in patients with *Hp* negative gastritis by the linear discriminant analysis effect size analysis[47].

Miao et al[48] studied the effect of *Hp* eradication in microbiota composition and found that GM profiles between *Hp* negative groups and previously *Hp* positive groups four months after successful eradication therapy were almost the same[48].

Table 2 shows the relative abundance of GM at phylum level among *Hp* positive and *Hp* negative patient groups. In particular, we present the minimum and the maximum values across the studies[36,42,43,47,48]. Also, we calculated the pooled percentages and the relative 95% confidence intervals. Among *Hp* positive patient groups, proteobacteria were more frequent, while among *Hp* negative patient groups, firmicutes and proteobacteria were more frequent.

IMPACT OF FACTORS ON GASTRIC MICROBIOTA COMPOSITION BEYOND *HP*-I

Beyond *Hp*, the composition of GM could be modified by some other factors, such as dietary habits, age, ethnicity, medication use and severity of gastric mucosa inflammation[18,27,49-53].

Proton pump inhibitor (PPI) raises the pH in the stomach, thereby altering the GM. Likewise, PPIs-driven gastric hypo-chlorhydria, can cause substantial changes in gut microbiota composition[54,55]. Two possible mechanisms, by which the mentioned PPIs can influence the GM composition, have been proposed: (1) By targeting directly bacterial and fungal proton pumps; and (2) By disturbing the natural gastric microenvironment through the gastric pH alkalization[56]. More specifically, GM of patients on PPIs therapy has more abundant bacteria compared to patients on H2RAs and untreated control. The composition of microbiota was quite similar to that of oropharyngeal or fecal bacteria[26]. Paroni Sterbini et al[57] showed a significant increase in the relative abundance of *Streptococcus* in patients taking PPIs irrespective of *Hp* status; they revealed that *Streptococcus* can be an independent indicator of the gastric microbiome changes in

dyspeptic patients secondary to the use of PPIs[57]. On the other hand, Parsons et al[40] by using 16S rRNA sequencing in gastric samples, showed that patients receiving PPIs had relatively few changes in the GM compared to healthy controls[39]. Besides, numerous reports indicated that the *Hp* moving from the antrum to body and fundus of the stomach is recorded particularly by long-term PPIs usage[58]. Thus, *Hp-I* eradication is proposed to patients received long-term PPI usage in order to prevent the proinflammatory trigger and thereby decreasing GC potential. Antibiotic ingestion also effects gastrointestinal microflora. Mason et al[59] revealed that treatment with cefoperazone caused changes in GM, with overgrowth of *Enterococci* and a decrease of *Lactobacilli*[59].

Attempting to correlate gastric mucosal inflammation with GM, an rise in *Streptococcus* and a reduction in *Prevotella* were found in patients with atrophic gastritis, *vs* healthy subjects[36]. Patients with autoimmune atrophic gastritis (AIG) exhibited a larger concentration of *Firmicutes* than patients with chronic atrophic gastritis (CAG) and a greater variety of microbial species than *Hp*-induced atrophic gastritis. This might be due to the differences in gastric acidity between the two conditions or additional factors such as their different immune profiles[39]. Researchers from Mexico, obtaining gastric tissue from patients with non-atrophic gastritis (NAG), IM and intestinal type GC through extraction of DNA for microbiota analyses using microarray methods, showed that bacterial diversity steadily decreased from NAG to IM to GC[59].

THE INTERACTION BETWEEN GASTRIC MICROBIOTA AND GASTRIC CANCER

The existence of multiple homeostasis mechanisms that take place in human stomach is a well-recognized phenomenon contributing to health maintenance by balancing the interaction between host gastric microbial diversity and mucosa-related factors[60,61]. When this balance is interrupted, a cascade of events occurs resulting in the emergence of inflammatory changes, dysbiosis and consequently, diseases including GC[36].

The mentioned hypochlorhydria appears to promote a decrease in microbial heterogeneity as well as the development of microorganisms which exhibit genotoxic

changes, and rising the ratio of nitrate to nitrite reductase microbe capacities implicated in gastric oncogenesis. Furthermore, the bacterial balance differentiates by rising the stomach pH, giving growth mostly of oral bacteria, such as *Streptococcus anginosus, Peptostreptococcus stomatis, Slackia exigua, Parvimonas micra* as well as *Dialister pneumosintes*. Such bacteria might play a role in GC progression via the induction of various metabolic pathways[62]. Thus, to improve the understanding of the influence of promoting the survival and spread of potentially genotoxic bacteria in the stomach and other GIT locations, it will be critical to describe the properties of the mentioned PPIs in GM composition. Nevertheless, no consensus exists regarding the role of PPIs in GC development. Based on a number of metanalyses and studies, there is an increased GC risk in patients using PPIs for a long time period[63] (~2.4 times more than non-users), despite *Hp* eradication[4,64,65].

Hp-I is a precise paradigm of the GM homeostasis disturbance sequelae[66]. The *Hp*-related inflammatory effects primarily act on the mucosal surface of the stomach variably affecting the production of mucin[67]. Differentiations of the latter seem to play a crucial role regarding the gastric carcinogenesis pathway[9]. Nevertheless, it should be stated that studies on the *Hp*-related mucin production changes have not yet been able to sort out whether this GC sequelae results in dysbiosis in the stomach or, conversely, to microbial diversity. These effects could be the backbone of GC development, given the fact that at the last stage of gastric malignancy oral or intestinal-type bacteria are predominantly discovered, something not seen in premalignant conditions (chronic gastritis, atrophy and IM) where *Hp* abundancy is more than clear. Whether this phenomenon is due to tumor-related mucin type differentiation, possibly resulting in GC-related microbiota must be elucidated[68].

As already stated, earlier studies have shown that *Hp* negative individuals exhibit a significant variability in microbiota composition which mainly consists of *Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes* and *Fusobacteria*. On the contrary, the stomach of *Hp* positive patients, is almost exclusively colonized by this infectious pathogen[42]. In line with this observation, it should be highlighted that from a specific point and beyond, the

GC progress seems not to be related with *Hp* presence, since the gastric adenocarcinoma microbiota mainly consists of intestinal and oral bacterial genera, and in addition this progression can happen even after successful *Hp* treatment (Figure 2)[67]. Similar findings emerged from the study by Yu et al[27] who investigated 160 individuals with gastric malignancy residing in China and Mexico. They showed that in the non-cancerous gastric regions, the *Hp* presence was significantly high, in contrast to the GC site with depletion even in the absence of *Hp*. The difference in microbiota diversity that patients with advanced malignant lesions exhibited, was further verified in many studies which revealed a marked presence of *Lactobacillus, Streptococcaceae, Staphylococcus, Clostridium* and *Fusobacterium* among others, underlying the crucial role that intestinal microbes play[63,69]. Lastly, Robinson et al[70] showed, after utilizing an advanced computer-based search algorithm, that GC was the second most diversely abundant neoplasm in terms of bacterial DNA molecules with dominant species highly comprising *Pseudomonas* and not *Hp*.

The above studies and their subsequent findings have been verified to an accountable level by well-designed animal model experiments, especially in C57BL/6 mice, where their stomach microbiota consisted of similar bacteria categories to those found in humans, namely *Firmicutes, Bacteroidetes, Proteobacteria* and *Actinobacteria*[71]. For instance, according to Lofgren et al[72], the *Hp*-related gastritis not only resulted in decreased GM variety (as seen in human individuals), but also significantly extended the interval to gastric malignancy emergence, especially when the only pathogen was *Hp*. The above interesting outcome was confirmed by the study of Lertpiriyapong et al[73], who showed that by adding even a small number of intestinal commensal pathogens to monocolonized by *Hp* germ-free insulin-gastrin (INS-GAS) transgenic mouse models' stomach, there was a progressive advancement to gastric neoplastic lesions.

Viewing the aforementioned data, while a role for *Hp* in gastric oncogenesis cannot be doubted, emerging data show that additional bacteria in the GM also seem to be involved in the transformation of stomach epithelial cells[74]. Nevertheless, whether it is the *Hp-I* that stimulates growth of unwanted bacteria or *vice versa* warrants clarification.

In a survey, Jo et al[75] showed that in GC patients, the records of nitrosating/nitrate-reducing microbes other than *Hp* were no less than doubled in comparison with healthy controls exhibiting similar *Hp* status, albeit insignificantly. Thus, further basic research is necessary, to illuminate whether GM alterations are crucial to GC development or are the result of alterations in the gastric setting.

Microbial infections have been incriminated for a variety of cancers, by transforming host cells and triggering neoplastic characters and inflammatory reactions, disrupting cell configuration and altering their genoms. Therefore, it is rational to consider the possible role of the intestine microbiota in gastric oncogenesis[76]. Furthermore, under the consideration that *Hp* plays a dominant role in Correa's cascade (NAG → atrophic gastritis → IM → dysplasia → GC), the inflammatory process of gastritis could be considered to be started and continued by *Hp-I*, which can colonize epithelium decades before neoplastic transformation. Ultimately, this transformation could develop owing to augmented pH of the stomach, because of the loss of parietal cells and the multiplication of microbes other than *Hp*[18]. Certainly, the microbiota differs between patients with chronic gastritis, IM and GC. The later indicates the significant role of gut microbiota in *Hp*-related tumorigenic effect. In contrast, progressive alterations in gastric pH could also be anticipated through *Hp* derived histological alterations, facilitating the gastric colonization from other bacteria[18]. Other investigators showed that the GC microbiota mainly included *Citrobacter, Achromobacter, Clostridium, Lactobacillus, Phyllobacterium* and *Rhodococcs*. Nevertheless, additional research is warranted to clarify the fingerprint of bacterial populations associated with gastric disorders, in connection with the Correa's cascade sequence.

Currently, the comprehension of dysbiosis-related genotoxicity and inflammation needs to move from descriptive studies to functionally based studies which investigate the effects of specific taxa and bacteria-derived metabolites on the gastric mucosa. In this regard, the potential introduction of probiotics should be studied thoroughly in order to delineate its effectiveness in the rebalance of human microbiota synthesis[77].

INTERACTION BETWEEN *HP*-I, GASTRIC MICROBIOTA AND GASTRIC CANCER

The perpetuation of *Hp*-I reduces microbiota diversity and is connected with atrophy, IM and GC[78]. Moreover, although it represents the main genus in chronic gastritis with a mean relative abundance of 42% (varying from 0.01%-95%), *Hp* presents a dramatic decrease in GC tissues with a relative abundance of 6%. In this regard, recent data based on RNA sequencing analyses, revealed that *Hp* entirely dominated the microbiota not only in infected patients but also in the majority of individuals categorized as *Hp*-uninfected using conventional approaches, thus implying an active role in all cases of GC development[78].

The vast majority of information regarding the role of GM in carcinogenesis derives from preclinical studies in INS-GAS transgenic mouse models. Complex microbiota has been associated with intensive gastric inflammation, epithelial damage, oxyntic gland atrophy, hyperplasia, metaplasia and dysplasia[71]. Moreover, co-infection with *Hp* in INS-GAS rodents predisposed to more severe gastric lesions and earlier development of early GC, in comparison to *Hp*-infected germ-free INS-GAS mice[71]. Concerning the co-infective bacteria, complex microbiota and restricted microbiota consisting of only three species of commensal murine bacteria (*Clostridium sp.*, *Lactobacillus murinus* and *Bacteroides sp.*) predisposed similarly to neoplasia generation in *Hp* positive models[73]. Further *in vivo* studies with *Hp*-I revealed that the co-infection with commensal microbiota accelerated the progression to gastric intraepithelial neoplasia and the progression to cancer, whereas the treatment with antibiotics delayed the gastric tumorigenesis in *Hp*-free and specific pathogen-free INS-GAS mice[73,79,80]. Moreover, the environment of gastric atrophy reduces the density of *Hp* aggregates to give rise to bacteria from other locations of the GIT, thus perpetuating the inflammatory process and genotoxicity, to induce malignant transformation. The overgrowth of such microbiome could partially contribute to the "point of no return" of carcinogenesis prevention after *Hp* eradication[81]. As already known, eradication of *Hp* is associated with a reduced risk of GC, although ambiguity exists over whether this is an isolated result from the

eradication of the *Hp* or the modification of the whole GM, as bacterial diversity increases probably beneficially[80].

Interestingly, Eun et al[82] reported variations in the composition and diversity of GM among patients with chronic gastritis, IM and GC. More specifically, in the early stages of carcinogenesis, *Hp* may trigger the development of CAG, rather than direct induction of GC[82]. Subsequently, the resulting increased pH, provokes changes in the constitution of GM, thus facilitating the progression from CAG to IM and finally to GC[83]. On the other hand, subjects with GC showed a significant increase in the *Bacilli* class and *Streptococcaceae* family, whereas the *Epsilonproteobacteria* class and *Helicobacteriaceae* family were decreased[82]. As suggested by Correa[84], chronic *Hp*-I triggers a CAG with the mentioned defective acid secretion, thus facilitating the excessive colonization of gastric micro-flora with bacteria capable of reducing nitrate to nitrite, to form N-nitroso compounds that are carcinogenic[84,85]. In this regard, the GC microbiome is different from atrophic gastritis and possesses increased representation of nitrate reductases, with *Citrobacter, Achromobacter, Clostridium Campylobacter, Deinococcus, Sulfurospirillum* and *Phyllobacterium* representing ascendant species[79], thus accelerating the development of GC following *Hp*-I in INS-GAS mice when compared to germ-free mice that were monocolonized by *Hp*[71]. Relatively, chronic treatment with the mentioned PPIs increases the potential of atrophy among *Hp* positive subjects[86], in contrast to *Hp* negative individuals or patients receiving eradication treatment, thus implying that the non-*Hp* microbiota could only promote gastric atrophy when co-existing with *Hp*[35,87].

The activity of gastritis is well known for its close relationship with *Hp*-I. A similar motif of diversity is suggested for further phyla, such as *Bacteroidetes* and increased abundances of *Firmicutes* or *Proteobacteria*, thus incriminating their dysbiosis for gastric carcinogenesis[87]. Nevertheless, despite the wide range of studies associating *Hp*-I with gastric dysbiosis, no data interpret the exact background of this interaction, which seems to promote a sustained inflammation and genotoxicity[88]. A widely acceptable pattern suggests that chronic gastric inflammatory response to *Hp* may modify the gastric environment, paving the way to the growth of a dysbiotic gastric bacterial community;

and *Hp* eradication reverses the gastric dysbiosis to a similar level to uninfected patients, and exerts beneficial effects on gut microbiota, achieving an increased probiotic and putative downregulation of drug-resistance[89]. More specifically, successful *Hp* eradication inhibited dysbiosis significantly ($P < 0.001$), although it remained higher than that of *Hp* negative arm ($P = 0.025$). Nonetheless, treatment failure was associated with increased dysbiosis rate, comparable to active *Hp*-I ($P = 0.351$)[89]. Intense dysbiosis was further found to be analogous to the progress from gastritis to atrophy, IM and GC (both $P < 0.001$)[89].

Pathophysiologically, the highly expressed VacA (vacuolating cytotoxin A), after *Hp*-I, binds to the receptor proteins tyrosine phosphatase α and β on gastric cells, thus generating pores to yield bacterial internalization[90]. Some data indicated that antibodies against VacA, could be correlated with both peptic ulcer and gastric malignant disorders, thus it could be considered as a biomarker of both pathologies[91]. Additionally, *Hp* survival promoted by VacA is independent of CagA (cytotoxin-associated gene A) accumulation. VacA is connected with mucolopin 1 (transient receptor channel) which impedes the death of microbial cells through autophagic procedure and permits the formation of an intracellular niche in which *Hp* survives[91]. In this regard, infection of the AGS gastric adenocarcinoma cell line with *Hp* for 6 h, leaded to autophagy that was dependent on VacA[92]. This implied that autophagy is activated by cells infected by *Hp* to evade the destructive effects of toxins, thus promoting cell survival. In addition, others reported that 1 d exposure to VacA disturbs the antiphagocytic signaling and accumulates defective autophagosomes in cells[92]. Likewise, *Hp* controls the autophagocytic pathway as well as the expression of genes related to autophagy in both macrophages and gastric epithelial cells[93]. Therefore, it appears that during the initiation of carcinogenesis, the aforementioned pathway has a regulatory role, and when suppressed, leads to premalignant disorders, induces oxidative stress, promotes cell growth, penetration, and eventually metastases. Concerning GC, this could lead to precursor lesions extension[93]. Interestingly, there is a direct association between

pathogens that induce dysbiosis and disturbed immune responses including apoptosis - autophagy and orodigestive cancers, including GC[93].

Besides, *Hp* releases a plethora of adhesins (BabA, BabB, SabA, AlpA and AlpB), which facilitate the opening of tight junctions (TJ) and adherent junctions (AJ)[94-96]. In this regard, *in vivo* CagA causes depolarization and disruption of the TJ barrier function in epithelial cells to the *Hp* attachment sites[7,94]. Additionally, after *in vitro* excessive administration, CagA binds to membrane e-cadherins, inhibits their interaction with β-catenin, to disrupt the AJs' integrity and tightness[97]. *In vivo* cagA with *Lactobacillus*, enhances the effect of *Hp* to human monocyte-derived dendritic cells (DC) leading to DC maturation and induction, beyond *Hp*, additional inflammatory mediators[93]. This implies that the bacteria that produce lactic acid could increase *Hp* related inflammation promoting gastric oncogenesis. The latter are in concordance with human GM studies displaying plethora of *Lactobacillus* in *Hp*-connected IM and GC (intestinal type) vs NAG[62] and the increased *Lactobacillus* in INS-GAS mouse model studies infected with *Hp* and reduced commensals (*Clostridium*, *Lactobacillus*, and *Bacteroides*) which develop gastric intraepithelial neoplasia[73]. Nevertheless, other findings indicate a probiotic *Lactobacillus* strain that inhibits *Hp* colonization in a Mongolian gerbil model[98]. More relevant to biofilm-associated *Hp*, *Streptococcus mitis* interacts with *Hp* in co-culture studies, converting it to coccoid cells, as proteomic analysis reveals, signifying an apparent impact on gastric oncogenesis linked with *Hp*[99,100]. Moreover, experimental data on INS-GAS mice co-colonized with *Hp* and *Streptococcus Salivarius* showed more severe gastritis when compared with solely *Hp-I* only at 5 months post-infection. The latter data signify strong interactions among several bacteria and *Hp* that in turn may affect *Hp*-related tumorigenesis[101]. Of note, *Hp*-induced biofilms are associated with resistance to *Hp* antibiotic eradication regimens[102]; *Hp* biofilms appear to be one of the main barriers to *Hp* eradication, by inhibiting antibiotics penetration, and augmenting the expression of efflux pumps and mutations, several therapeutic failures, and chronic infections[103].

Finally, the interplay between *Hp* and GM in the pathogenesis of GC can be dependent on Toll-like receptors, through a perpetual stimulation by *Hp* and potentially by other microorganisms[104]. In this regard, *Hp*-I seems to create a premalignant environment of atrophy and IM, and the subsequent alterations in GM in later stages play a more relevant role in carcinogenesis itself[105].

CONCLUSION

It is more than clear that *Hp*-I, GM and GC constitute a challenging tangle due to the strong interaction between them making it difficult to unroll it.

The stomach harbors a large and diverse bacterial community with *Hp*, a member of Proteobacteria phylum, being the most dominant and abundant genus. The main phyla colonizing stomach are Proteobacteria, Bacteroidetes, Firmicutes, Fusobacteria and Actinobacteria. Most studies show that *Hp* has inhibitory effects on the colonization of other bacteria, harboring a lower diversity of them in the stomach. Other factors that influence GM are dietary habits, age, ethnicity, medication use (PPIs, antibiotics), gastric mucosa inflammation and GC. It is worthwhile to mention that GM differs in patients with chronic gastritis, IM, dysplasia or GC, but its role in GC has not yet been fully elucidated. Data show that from a specific point and beyond, apart from *Hp*-related gastritis, the GC progress seems not to be related with *Hp* presence, since the gastric adenocarcinoma microbiota mainly consists of intestinal and oral bacterial genera, considering that this progression can happen even after successful *Hp* eradication. The above has been verified to an accountable level by well-designed animal model experiments. In accordance, beyond *Hp*'s role in gastric oncogenesis, other bacteria, *Hp*-stimulated or not, in GM also seem to be responsible for transformation of gastric epithelial cells.

To conclude, the aforementioned studies amongst others have begun to shed light into the maze of GC complex pathogenesis where abundant data show that beyond *Hp* related gastritis, additional pathogens might contribute to this type of cancer development. Nevertheless, large-scale experiments are needed to discern the exact role of different

kinds of pathogens which reside in the stomach and their contribution to neoplasia emergence, aiding in the prediction of adverse prognosis of a specific microbiota diversity. Only then would the manipulation of GM be feasible, modifying the number and the types of the necessary commensals.

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