EDITORIAL

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How does bacterial pathogen *Helicobacter pylori* control responses to cellular stress?

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“During viral and bacterial infections, one of the biggest challenges for the host cells is to assure their genomic integrity. *Helicobacter pylori* was found to cause several types of DNA damage, including single and double strand DNA breaks.”

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Helicobacter pylori is a Gram-negative bacterial pathogen that infects more than half of the world's population. During a long coevolution with the human host, the bacteria have adapted to live in the acidic environment of the human stomach. Without treatment, *H. pylori* can persist for many decades or potentially for the entire human lifetime, typically causing asymptomatic gastritis. In some infected individuals, however, *H. pylori* may lead to more severe consequences, such as gastric atrophy, peptic ulcer and cancer. *H. pylori* infection has been recognized as the strongest known risk factor for the development of gastric adenocarcinoma. The etiology of gastric cancer is complex, comprising bacterial variability, host genetic and environmental factors. Recent research demonstrated that interactions between virulence and host factors are pivotal for understanding of pathological changes caused by *H. pylori* infection. Among bacterial virulence factors of special interest are cytotoxin-associated gene A (CagA) and vacuolating

cytotoxin A (VacA). CagA is a large bacterial protein that is injected into the host cells upon bacterial attachment through a syringe-like pilus structure termed the type IV secretion system. Following injection, CagA is rapidly phosphorylated and interacts with multiple host proteins causing profound alterations of the host cellular signaling pathways, including activation of multiple oncogenes and cytoskeletal rearrangement [1]. Among the major oncogenic pathways activated by CagA are Ras/Erk, PI3K/Akt and Wnt/ β -catenin [2]. CagA is itself considered to be an oncoprotein, since it has the ability to induce development of gastrointestinal and hematopoietic tumors in laboratory animals. VacA protein is another important pathogenic factor for *H. pylori*. It is secreted as a soluble protein into the extracellular space and is able to form membrane channels in host cells. VacA is known to contribute to bacterial colonization, virulence and the development of peptic ulcers and gastric cancer (reviewed in [3]).

KEYWORDS

- gastric cancer • *Helicobacter pylori*
- p53

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During viral and bacterial infections, one of the biggest challenges for the host cells is to assure their genomic integrity. *H. pylori* was found to cause several types of DNA damage, including single and double strand DNA breaks (DSBs). It also increases the mutation rate and chromosomal instability. One clear source of DNA damage is reactive radicals. *H. pylori* was found to induce reactive oxygen species (ROS), reactive nitrogen species (RNS) and lipid peroxidation in the human stomach. The most commonly produced base lesion by ROS, and the one most often measured as an index of oxidative DNA damage, is 8-hydroxyguanosine. Several studies demonstrated increased levels of 8-oxoguanine lesions during *H. pylori* infection. The main source of ROS/RNS production is possibly the host immune cells, but *H. pylori* itself can produce ROS, such as superoxide anion (O_2^-). Interestingly, recent high-throughput genomic analyses show that the *H. pylori* causes a specific pattern of DNA damage, targeting the transcribing and telomere-proximal regions correlating with already described genomic alterations typical for gastric cancer [4]. These findings strongly support and emphasize the role of *H. pylori* induced DNA damage in gastric carcinogenesis.

As a result of DNA damage, *H. pylori* infection leads to activation of the DNA damage checkpoint and DNA damage repair. Early studies have identified the activation of ataxia telangiectasia mutated (ATM), recruitment of 53BP1 and MDC1 proteins as well as phosphorylation of histone H2AX, a well-known marker for DSBs [5]. Activation of the ATM-dependent DNA damage response was confirmed in specimens collected from humans infected with *H. pylori* [6]. However, in conditions of chronic infection, DNA damage repair appears to be insufficient to completely repair DNA damage causing mutations and chromosomal instability. Moreover, it has been demonstrated that *H. pylori* systematically reduces/modulates multiple cellular pathways such as base excision repair (BER), mismatch repair (MMR), non-homologous end-joining (NHEJ) and homologous recombination (HR) involved in DNA damage repair (reviewed in [7]). The latter two repair pathways (NHEJ and HR) are responsible for repair of DSBs, the most severe type of DNA damage. A number of proteins involved in DNA damage repair were found to be downregulated by *H. pylori*. It was proposed that during *H. pylori* infection, there is a shift in repair of DNA DSBs from HR to

NHEJ known to be more susceptible to errors, contributing to genetic instability in infected cells [4].

The key component of DNA damage response is the p53 tumor suppressor that has earned the name ‘the guardian of the genome’. p53 protein is directly regulated by ATM, ATR (ATM- and Rad3-related) and other kinases involved in the DNA damage response network. It can be considered a key manager of ‘the executive branch’ of the DNA damage response machine. p53 exerts its main function as a transcription factor affecting expression of various target genes involved in regulation of cell cycle, apoptosis and senescence. In normal conditions, p53 has a short half-life as a consequence of its ubiquitination by the E3 ubiquitin ligase, MDM2, causing its degradation in the proteasomes. It was shown that *H. pylori* has the ability to modulate protein levels of p53, causing its degradation after infection, putting p53 in the focus as a principal target of *H. pylori* induced carcinogenesis [8–10]. Degradation of p53 is a consequence of activation of MDM2 by the Akt and Erk kinases by *H. pylori* [11,12]. CagA was found to induce degradation of p53. The impact of *H. pylori* on p53 could be especially important in gastric stem cells. It was shown that CagA is more stable in those cells while in other tumor cells it is degraded by autophagy [13]. Regulation of p53 levels by *H. pylori* appears to be very dynamic and exerted through various mediators. Experiments in Mongolian gerbils discovered a bimodal change in the p53 protein levels after *H. pylori* infection. p53 level was first increased upon initial infection due to stress, then it is rapidly decreased to reach a second peak of expression several weeks later. The latter peak is concomitant with the development of gastritis and inflammation-driven DNA damage [14]. These findings led to a hypothesis that at a certain time, levels of p53 reflect a balance between p53 degradation induced by bacteria and p53 induction caused by cellular stress [14].

In the last decade, several p53 isoforms have been described that are produced by the alternative p53 gene promoter and alternative splicing, some of them even have potential oncogenic functions, in contrast to wild-type p53 [15]. Notably, our data revealed an increase in expression of p53 isoforms, $\Delta 133p53$ and $\Delta 160p53$ by *H. pylori*. These isoforms have a dominant-negative effect on wild-type p53 protein [12]. $\Delta 133p53$ is also known to act as a dominant-negative

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inhibitor of p53, another member of the p53 protein family, level of which is increased after *H. pylori* infection [10].

p14ARF (alternative reading frame; p19ARF in rodents) tumor suppressor, a product of the *CDKN2A* locus, was found to regulate stability of p53 protein during *H. pylori* infection. ARF positively regulates the p53 activity through its inhibition of MDM2 and Mule/ARF-BP1 E3-ubiquitin ligases that are shown to contribute to p53 degradation during infection. ARF was shown to be a critical determinant of p53 protein levels in infected cells. In cell lines with higher ARF levels, p53 was more resistant to degradation caused by infection, while in the cells which for various reasons had low ARF levels, rapid decrease in p53 was observed. The importance of the modulation of p53 levels by *H. pylori* was further emphasized by the observation that clinical isolates of *H. pylori* from high-risk gastric cancer areas had a greater ability to decrease p53 levels compared with ones from low-risk areas [16].

Currently, it became clear that *H. pylori* is not unique among bacterial species in its ability to regulate p53. *Shigella flexneri*, *Chlamydia trachomatis* and potentially other *Chlamydia* species were recently found to induce degradation of p53 protein [17,18]. Interestingly, degradation of p53 occurs through a similar mechanism earlier described for *H. pylori*. These bacteria were found to induce the PI3K/Akt/MDM2 pathway leading to degradation of p53. It was also reported that bacterial degradation of p53 interferes with the host's response to genotoxic stress and may contribute to tumorigenesis. It is plausible to suggest that inhibition of p53 activity by bacterial pathogens is more common than one may think,

the distinguishing factor between various bacterial species may be the specific purpose for which p53 is being targeted by a specific pathogen.

In conclusion, *H. pylori* infection causes damage of DNA in host cells, triggering the DNA damage response. Inhibition of DNA damage repair and p53 activity by *H. pylori* may be the links in the same chain of bacterial adaptive responses directed to prevent the host response to cellular stress. Unfortunately, this may have severe consequences for the host, increasing the risk of pathological changes and tumor development. Future studies of bacterial modulation of host stress responses will lead to better understanding of bacterial pathogenesis and help to identify potential targets in the prevention of cancers associated with bacterial infections.

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