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Pathogenic enablers — toxic relationships in the stomach

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Abstract

Chronic infection with *Helicobacter pylori* is the strongest known risk factor for the development of gastric cancer. Saju *et al.* shed new light on mechanisms by which Epstein–Barr virus, a viral initiator of gastric cancer, potentiates the oncogenic effects of *Helicobacter pylori* in the stomach.

Worldwide, gastric cancer is the third leading cause of cancer-related mortality, accounting for >700,000 deaths each year¹. The most common type of gastric cancer is adenocarcinoma, which can be stratified histologically into two distinct variants: diffuse-type gastric cancer, which consists of individually infiltrating neoplastic cells that do not form glandular structures; and intestinal-type adenocarcinoma, which progresses through a series of well-defined histological steps². In 2014, a molecular classification of gastric cancer was developed through the Cancer Genome Atlas study that comprehensively analysed nearly 300 primary gastric adenocarcinomas and segregated tumours into four molecular subtypes: Epstein–Barr virus (EBV)-positive tumours; microsatellite unstable tumours; genomically stable tumours associated with diffuse-type histology; and intestinal-type tumours with chromosomal instability³. However, the degree to which these molecular classes overlap is unknown.

The strongest known environmental risk factor for developing gastric cancer is prolonged colonization with *Helicobacter pylori*, and microbial virulence constituents clearly modify disease risk⁴. *H. pylori* strains that possess the *cag* island — a type IV secretion system that translocates its substrate, CagA, into host cells — augment the risk of gastric cancer compared with *cag*[−] strains⁴. Following translocation, CagA becomes tyrosine phosphorylated at C-terminal glutamate-proline-isoleucine-tyrosine-alanine (EPIYA) motifs, endowing CagA with oncogenic activity⁵. In addition to *H. pylori*, EBV, a ubiquitous human herpes virus with oncogenic potential, is associated with the development of gastric adenocarcinoma, and has been linked to ~9% of gastric cancer cases worldwide⁶. An exciting new study by Saju *et al.*⁷ now describes a paradigm of how a bacterium and a virus collaborate to potentially exacerbate a disease they can each cause independently.

Specifically, Saju *et al.*⁷ report that a host phosphatase, SHP1 (also known as tyrosine-protein phosphatase non-receptor type 6 or PTPN6), interacts with *H. pylori*-delivered CagA

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Competing interest statement

The authors declare no competing interests.

and functions as a negative regulator of CagA phosphorylation. Using a combination of transient CagA transfections and co-culture of viable *H. pylori* *cag*⁺ strains with gastric epithelial cells, the researchers demonstrate that CagA forms a complex with SHP1, which was independent of CagA tyrosine phosphorylation status and did not involve CagA EPIYA phosphorylation motifs⁷. Glutathione *S*-transferase pull-down assays verified the direct interaction between CagA and SHP1, which required the *N*-terminal region of SHP1, and both the *C*-terminal and *N*-terminal regions of CagA⁷.

In terms of downstream effects, levels of phosphorylated CagA were decreased when SHP1 was ectopically overexpressed in host cells, which required SHP1 phosphatase activity. Conversely, levels of phosphorylated CagA were increased by knockdown of SHP1 (via small-interfering RNA), suggesting that SHP1 is a rheostat that tightly regulates the level of CagA tyrosine phosphorylation⁷. To definitively determine whether SHP1 is the mammalian phosphatase that dephosphorylates CagA, Saju *et al.*⁷ used an *in vitro* phosphatase assay whereby SHP1 was co-administered with tyrosine-phosphorylated CagA. Under these conditions, CagA was dephosphorylated by SHP1 and the active phosphatase domain was indispensable. Notably, under the same conditions, the related phosphatase SHP2 had no effect on the phosphorylation status of CagA but was instead, activated⁷. Collectively, these elegant experiments demonstrate that SHP1 is activated upon complex formation with CagA and this interaction induces CagA dephosphorylation, subsequently reducing the oncogenic potential of phosphorylated CagA⁷.

“... a paradigm of how a bacterium and a virus collaborate to potentially exacerbate a disease they can each cause independently”

As co-infection with *H. pylori* and EBV is present in the majority of EBV-positive gastric cancer specimens, Saju *et al.*⁷ next demonstrated that EBV is capable of hyper-methylating the SHP1 promoter (FIG. 1), thus decreasing expression of SHP1 at both the RNA and protein level. Importantly, the oncogenic potential of phosphorylated CagA was enhanced in the presence of EBV, indicating a novel role for this virus in promoting pathways that might ultimately lead to gastric carcinogenesis⁷.

Identification of synergy between *H. pylori* and EBV has important implications for managing patient populations and identifying individuals at high risk of developing gastric cancer. To date, it is not possible to accurately predict which *H. pylori*-infected individuals will develop this malignancy, and because the vast majority of infected individuals do not develop cancer, it is imperative to develop noninvasive tests with strong predictive potential. On the basis of this new study⁷, seropositivity for both *H. pylori* and EBV could help identify such a high-risk population. However, the molecular interaction between EBV and CagA is likely to be uncommon within the collective context of gastric adenocarcinoma cases. Only a minority of gastric cancers (<10%) harbour EBV and not all strains of *H. pylori* are CagA positive⁴. Nevertheless, these results invoke a novel working model for how bacteria and viruses might interact within and exogenous to the gastric epithelium.

In addition to bacterial–viral interactions, there is clearly precedence for microbial collaborations augmenting disease within the stomach. In germ-free mice that are monocolonized with *H. pylori*, gastric cancer develops at a markedly attenuated rate

compared with mice that harbour a more complex gastric microbiota of at least three specific species of commensal bacteria: ASF356 *Clostridium* species, ASF361 *Lactobacillus murinus* and ASF519 *Bacteroides species*^{8,9}. These effects could be magnified even further if progenitor cells are targeted by *H. pylori*; investigations have demonstrated that levels of CagA are increased in *H. pylori*-infected cancer stem-like cells when compared with infected differentiated cells¹⁰.

Taken together, the work of Saju and colleagues⁷ provides a fascinating example of how a virus and bacterial species collaborate within the gastric epithelium to exacerbate progression towards cancer. However, there are still many questions to address regarding the pathobiology of *H. pylori* and EBV infection, alone or in combination, and the mechanisms through which each pathogen promotes the cascade to carcinogenesis.

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Figure 1. Co-infection with *Helicobacter pylori* and EBV in gastric epithelial cells promotes the oncogenic activity of CagA

CagA is translocated into host cells by the *H. pylori* *cag* secretion system where it is tyrosine phosphorylated by Src and Abl kinases. **a** | In the absence of Epstein–Barr virus (EBV), SHP1 interacts with CagA and negatively regulates CagA phosphorylation. **b** | With concomitant EBV infection, the SHP1 promoter is hypermethylated and its expression is downregulated. This process results in elevated levels of oncogenic phosphorylated CagA.