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Gained in translation: the importance of biologically relevant models of *Helicobacter pylori*-induced gastric cancer

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The attributable risk of gastric cancer conferred by *Helicobacter pylori* ranges from 75% to more than 90% depending on *H pylori* prevalence; however, only a fraction of colonised persons ever develop neoplasia.¹² Disease risk involves well-choreographed interactions between pathogen and host, which are dependent upon strain-specific bacterial factors as well as host genotypic traits, each of which can be amplified by the environment. In their paper published in *Gut*, Kwon *et al* provide fresh insights into the role of a tumour suppressor, vitamin D3 upregulated protein 1 (VDUP1), in *H pylori*-associated gastric carcinogenesis (*see page 53*).³ Importantly, these investigators used multiple model systems to demonstrate that VDUP1 negatively regulates carcinogenesis induced by the combination of *H pylori* and N-methyl-N-nitrosourea (MNU) via dissociating proliferation from apoptosis and suppression of tumour necrosis factor α (TNF α)-dependent nuclear factor κ B (NF- κ B) activation and subsequent cyclo-oxygenase-2 (COX-2) expression.

Using a genetic model of VDUP1 deficiency, Kwon and colleagues observed that the incidence and severity of gastric premalignant and malignant lesions was increased in *vdup1*^{-/-} mice compared with wild-type mice.³ This was accompanied by hyperproliferation that was not balanced by a corresponding increase in apoptosis. Contact between *H pylori* and epithelial cells in vitro dysregulates signalling pathways that influence oncogenesis, which mirrors interactions between *H pylori* and epithelial cells that occur within infected tissue. Kwon *et al* therefore also used an in vitro model of microbial:epithelial contact to define specific mechanisms through which loss of VDUP1 may augment carcinogenesis. In human AGS gastric epithelial cells, overexpression of VDUP1 suppressed epithelial proliferation, similar to results derived from the in vivo experiments. Delving into mechanisms on a deeper level, the authors then examined the effects of overexpression of VDUP1 on signalling pathways that had previously been shown to be dysregulated by this receptor and demonstrated that VDUP1 suppressed TNF α expression, NF- κ B activation and production of COX-2. The authors then returned to their mouse model of carcinogenesis to corroborate the in vitro findings by demonstration that VDUP1 knock-out mice responded to

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exposure with *H pylori* and MNU with significantly higher TNF α , activated NF- κ B and COX-2 expression levels than wild-type mice.³ These results highlight the role of VDUP1 as a novel tumour suppressor within the context of gastric carcinogenesis and also emphasise the critical importance of thoughtful oscillation between systems to recapitulate more fully the events occurring within the gastric niche.

Animal model systems have provided valuable insights into the host, bacterial and environmental factors involved in gastric carcinogenesis. Rodents and primates represent the primary models that have been used and, although each model has its own distinct advantages and disadvantages, they should be viewed as complementary systems. Mice are inbred, permitting host variables to be carefully controlled, although most wild-type strains of mice develop only mild inflammation and not cancer following *H pylori* infection alone, thus necessitating the use of multiple agents such as *H pylori* and MNU to induce gastric cancer, as in the current study. Mongolian gerbils are outbred and are not as useful as mice for the study of host factors, but gerbils can develop cancer when colonised with certain strains of *H pylori*, even in the absence of other carcinogenic factors. Thus, gerbil experiments provided crucial supportive evidence for the World Health Organization classification of *H pylori* as a true carcinogen, and the gerbil model provides a useful system to study the impact of other factors such as acid output on gastric carcinogenesis.⁴⁵ Primates are the most closely related of these models to the human host, but experimental manipulations in monkeys cannot be conducted on the same scale as rodents and, therefore, large studies are often impractical due to costs.

Another advantage of animal model systems is that the effects of additional endogenous elements such as the gastrointestinal microbiota on *H pylori*-induced injury can be studied in depth. The use of broad range 16S rRNA PCR coupled with high throughput sequencing has demonstrated that *H pylori* does not simply exist as a monoculture within the human stomach but, instead, is a resident of a distinct gastric microbial ecosystem. While *H pylori* is the dominant species, the presence of other microorganisms in the stomach provides a genetic repository which may facilitate the generation of novel traits that influence gastric carcinogenesis, raising the question whether the presence of a co-existing gastric microbiota alters the cancer risk induced by *H pylori*. Lofgren *et al* recently performed an elegant study to address this question in which specific pathogen-free (SPF) or germ-free mice with an enhanced propensity to develop gastric cancer (hypergastrinaemic INS-GAS mice) were infected with *H pylori*.⁶ Their results clearly demonstrated that the presence of a gastric microflora enhanced the development of gastric intraepithelial neoplasia in *H pylori*-infected SPF compared with *H pylori*-infected germ-free mice at 7 months post-challenge. Furthermore, *H pylori* colonisation altered the gastric microbiota of SPF mice by enriching the proportion of Firmicutes and reducing the proportion of Bacteroidetes. These data provide a potential explanation for intriguing previous work demonstrating that *H pylori* can induce the production of antimicrobial defence molecules which may function to sculpt the gastric microbial ecosystem towards a more favourable niche that can support long-term survival of *H pylori*.

Animal models have also provided important insights into the extragastric microbial influences that may impact on disease in the stomach. Lemke *et al* have shown that concurrent intestinal *Helicobacter* infections can affect the severity of *H pylori*-induced injury in the stomach.⁸ Using a dual coinfection model, these authors found that precolonisation of mice with the intestinal *Helicobacter bilis* prior to *H pylori* challenge significantly attenuated gastric inflammation and injury compared with mice challenged with *H pylori* alone. More recent work from this same group has determined that these effects are strain-specific in that precolonisation with the intestinal *Helicobacter muridarum*, similar to *H bilis*, attenuated gastric injury induced by *H pylori*.⁹ However, preinfection with

Helicobacter hepaticus reciprocally augmented the severity of *H. pylori*-induced damage within the stomach. These effects may be mediated by differential activation of interleukin 17, as levels of this cytokine were increased by *H. hepaticus* but reduced by *H. muridarum*, implicating the intestinal microbiota as a biological rheostat poised to dampen or amplify microbially-induced injury in the stomach.

In summary, informative and tractable animal models that are colonised by well-defined microbial pathogens represent ideal systems for the study of complex human disorders such as gastric cancer and have provided an invaluable resource to permit the development of a focused approach to selectively target human populations at high risk for disease. In addition to defining mechanisms through which *H. pylori* mediates carcinogenesis, investigations that focus on model organisms colonised by this pathogen may also construct a paradigm for other cancers that arise from inflammatory foci within the gastrointestinal tract.

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