Gastric models

Dr Yana Zavros describes a groundbreaking new cell culture technique that enables a biologically realistic exploration of a bacteria-induced cancer while avoiding the confounding host immune response.

Could you begin by outlining how your previous work guided your current research on gastric cancers?

My research interest in the regulation of the host innate immune response and its role in the development of gastric cancer began during my postdoctoral training in the laboratory of Dr Juanita Merchant at the University of Michigan. I applied the knowledge of the physiology and biology of gastrointestinal peptides that I gained as a graduate student at the University of Melbourne, Australia, under the mentorship of Dr Arthur Shulkes, to understand the regulation of gastrin and somatostatin during Helicobacter pylori infection. This also gave me the tools to identify the mechanisms by which bacterial infection initiates the development of gastric cancer.

The objective of our current research is to identify the underlying mechanism by which H. pylori-host interactions trigger the disruption of epithelial cell differentiation and thus the cascade leading to cancer. The acquisition of such knowledge is the first step in the continuum of research required to achieve our long-term goal: to understand the pathogenesis of H. pylori-induced gastric cancer.

Why does your research specifically focus on H. pylori pathogenesis and its effect on cancer progression?

The severity and localisation of the inflammation that results from H. pylori infection is believed to dictate the pathological consequence of disease. Individuals most at risk of developing gastric cancer are those in whom the bacteria colonise the corpus (main body) or fundus (curved upper section) of the stomach when acid secretion is impaired. The subsequent development of severe inflammation in the corpus leads to atrophy and metaplasia. Our research uses human- and mouse-derived fundic/corpus gastric organoid cultures, which allow us to assay changes in gastric epithelial cell proliferation and differentiation in relation to direct interaction with H. pylori.

What are these gastric organoids and why are they useful models?

Our knowledge of H. pylori pathogenesis has been predominantly based on data generated from gastric cancer cell lines or in vivo animal models of inflammation. The limitations in acquiring such knowledge have therefore been attributed to the inability to evaluate molecular mechanisms of bacterial and host cell interactions in a setting of a sustained gastric epithelial cell diversity and polarity. Our current work reports the development and use of a novel model of primary human and mouse cultured gastric epithelial cells that are organised into 3D spheroid units containing a lumen, known as gastric organoids. These models recapitulate key features of the gastric environment, including the presence of the major gastric cell lineages and a polarised epithelium containing functional parietal cells that secrete gastric acid. In particular, the human-derived fundic gastric organoid culture system represents a vital new technique for modelling H. pylori infection within normal human tissue in vitro.

Our study takes advantage of the presence of the defined lumen in these models, which allows us to inject live H. pylori directly into the gastric organoid and assay the epithelial response without the influence of host-recruited factors. This contribution is significant because it provides the knowledge required to potentially develop techniques that disrupt bacterial colonisation and prevent disease progression.

How do your collaborators contribute to the realisation of your research aims?

Dr Micheal Helmrath at the Cincinnati Children’s Hospital Medical Center, Ohio, has extensive experience using protocols to isolate and expand intestinal glands, known as crypts, from both mice and humans. He has been responsible for the sleeve gastrectomies and collection of the human fundic tissue from which the gastric organoids are derived. In Dr Helmrath’s laboratory, Dr Maxime Mahe has assisted us with the development and culture of human fundic organoids.

Do you see this work translating into clinical practice?

The rationale for these studies is to acquire an understanding of the molecular mechanisms by which H. pylori infection induces disease. Acquiring such knowledge may give us the ability to develop techniques that disrupt bacterial colonisation and thus prevent disease progression. In addition, our studies show the feasibility and potential for the transplantation of human-derived organoids from human primary tissue, and provide information for the development of new regenerative strategies to treat gastric tissue damage.
Hard to stomach

*Helicobacter pylori* has been implicated in chronic gastritis and stomach cancer. Now, pioneering research into this common gastrointestinal bacterium taking place at the University of Cincinnati, Ohio, is revealing new mechanisms and targets for clinical treatment of these conditions.

**OVER HALF OF** the world’s population is infected with *Helicobacter pylori*, a bacterium most commonly found in the upper gastrointestinal tract. In the majority of people, this infection is asymptomatic, however, it has the potential to cause gastritis, a painful inflammation of the stomach lining that promotes ulcer development and can increase the risk of stomach cancer.

Chronic gastritis caused by *H. pylori* infection leads to atrophy of the stomach lining, wherein the acid-secreting parietal cells are replaced by mucus-secreting cells – a response that often results in abnormal cellular growth and the progression of tumour development. Understanding the interaction between *H. pylori* and stomach cells has been difficult, however; the host immune system and natural environment introduce confounding effects into the investigation, while *in vitro* cell cultures are typically not biologically realistic enough to provide useful data for this phenomenon.

At the University of Cincinnati, Dr Yana Zavros is pioneering the development of new approaches to understand how *H. pylori* infection can induce gastritis and cancer in the stomach: “Prolonged cell proliferation in the gastric mucosa is a precursor to the progression from chronic inflammation to gastric cancer in response to *H. pylori* infection,” she explains. “However, the mechanism by which this bacterium induces epithelial cell proliferation is not well defined.” To properly investigate this issue, her team has developed a novel, more natural type of cell culture that is allowing them to shed new light on the interactions between host cells and pathogenic strains of *H. pylori*.

**REALISTIC CELL CULTURES**

Zavros and her colleagues cultured primary human- and mouse-derived gastric epithelial cells as 3D structures called gastric organoids. These innovative structures contain functional cells enclosing an internal lumen, providing a more realistic environment to directly investigate the interaction between *H. pylori* and the gastric cells. Organoids open up several new avenues of research, enabling these researchers to understand gastric biology, epithelial cell differentiation and how colonisation by these bacteria alters healthy physiology. "With the development of this culture system we are able to identify the direct impact of bacterial infection on the gastric epithelium, independent of the host’s immune response," summarises Zavros.

The pathogenicity of *H. pylori* is enhanced in strains containing the cytotoxin-associated gene (Cag) pathogenicity island, a section of the genome that leads to a stronger inflammation response in the stomach during infection. The bacterium secretes the CagA protein into the epithelial cells lining the stomach and it becomes activated by the tyrosine kinase c-Met receptor, stimulating cell signalling and proliferation. Zavros began by investigating whether specific cells were targeted by the bacteria, and discovered that cells coated with the surface molecule CD44 were important for *H. pylori*-induced epithelial cell proliferation. **CD44 is a...**

**Increased gastric organoid proliferation, as marked by 5-ethynyl-2’-deoxyuridine (EdU) staining, in response to *H. pylori* infection within 24 hours of microinjection.**
INTELLIGENCE

USING GASTRIC ORGANIODS TO UNDERSTAND HELICOBACTER PYLORI-INDUCED GASTRITIS AND GASTRIC CANCER

OBJECTIVES
- To explore the role played by the cytotoxin-associated gene (Cag) pathogenicity island in Helicobacter pylori and the CD44 cell surface receptor in H. pylori-induced epithelial proliferation and subsequent gastric metaplasia
- To employ gastric organoids as effective models for gastric epithelial cells in order to advance treatments for gastric cancer and gastritis

KEY COLLABORATORS
Nina Bertaux-Skeirik, Michael A Schumacher, Amy C Engevik, Jing Li, University of Cincinnati, USA • Professor Micheal Helmrahan, Maxime Mahe, PhD; Rui Feng, Cincinnati Children's Hospital Medical Center, USA • Richard M Peek Jr, MD, Vanderbilt University, USA • Professor Karen Ottmann, University of California Santa Cruz, USA • Dr Véronique Orian-Rousseau, Karlsruhe Institute of Technology, Germany • Professor Greg P Boivin, Wright State University and Veterans Affairs Medical Center, USA

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YANA ZAVROS completed her PhD at the University of Melbourne, Australia, which marked the beginning of her research career exploring the role of regulatory peptides in gastric biology, physiology and pathophysiology.

HEDGEHOG GENE FAMILY
Sonic hedgehog (Shh) is a gene known to be crucial in organogenesis of the developing embryo, but it is also important in the maintenance of gastric epithelial differentiation and function, and may be involved in gastric cancers when dysregulated. The loss of Shh induces stomach atrophy and the removal of acid-secreting parietal cells, in a similar process to that which occurs during chronic inflammation. In an intriguing development, Zavros discovered that gastrin, the hormone responsible for stimulating parietal cells to produce acid and sustaining their differentiated state, regulates Shh expression. This linked Shh to gastrin as two important regulators of parietal cell differentiation; if either is removed, the stomach atrophies and parietal cells are lost, leading to epidermal cell proliferation and potentially gastric cancer.

The loss of Shh from parietal cells also results in hypergastrinaemia, an overproduction of gastrin. This is accompanied by a hyperproliferation of surface mucus-secreting cells and an increased expression of the Shh-related gene Indian hedgehog (Ihh). The Cincinnati group showed that Ihh mediates the gastrin-induced proliferation of epithelial cells in the stomachs of adult mice.

H. pylori induces Shh expression from the acid-secreting parietal cells; however, until recently the mechanisms behind this response were unclear. H. pylori infection is known to activate NFkB signalling, which leads to Shh expression and dysregulation. Zavros applied the mouse gastric organoid approach to the problem, which removed the confounding effects of in vivo studies, as well as revealing that this mechanism does indeed occur in these functional cells and, importantly, that it is CagA dependent. This suggests CagA as a potential target for the development of therapeutics in the future.

REPAIRING GASTRIC DAMAGE
Amazingly, gastric organoids also show potential for being directly employed in the treatment of chronic gastritis. As the body ages, the stomach increasingly loses the ability to repair itself, leading to inflammation and susceptibility to chronic ulceration. Zavros showed that it is possible to transplant both mouse- and human-atriated gastric organoids into the stomach epithelia of aged mice, promoting the regeneration of the damaged tissue and healing of gastric ulcers.

The future clinical benefits of these findings are exciting, as they provide crucial information to advance the field of regenerative medicine. The team will now shift some of its focus to the regenerative capacity of human-derived organoids, with the ultimate goal of informing the development of strategies to treat the gastric tissue damage that results from H. pylori-induced gastric disease.

Gastric organoids have already improved our understanding of when and how gastritis and stomach cancers can develop, as well as suggesting possible targets for the development of therapeutics. In addition, their ability to regenerate damaged gastric tissue in mice has opened up another exciting avenue of research. Zavros and her team are still exploring the diverse range of organoid uses, investigating gastric biology and the effects of H. pylori colonisation on epithelial cell differentiation. “Our contribution is significant because it is expected to provide knowledge required to potentially develop techniques to disrupt bacterial colonisation and prevent disease progression,” states Zavros, who is now hoping to use the organoids to evaluate the virulence of other pathogenic H. pylori strains on the stomach epithelium. Different strains of bacteria isolated from patients could conceivably be directly tested for their ability to increase the risk of cancer development. This deep understanding of H. pylori pathogenicity has the potential to revolutionise the prevention of colonisation and ultimately reduce the incidence of gastric cancer.