IBD

BACTERIOPHAGE VIROME IN IBD

Few data exist on the role of intestinal bacteriophages in IBD. New findings reveal alterations of the phage population and elevation of phages that infect microbiota with pathogenic potential (pathobionts) in a mouse model of ulcerative colitis. Furthermore, the phageome of mice overlapped with that of humans in both healthy and diseased states.

In their study, Duerkop et al. examined the faecal metagenome of mice with chronic colonic inflammation induced by adoptive T cell transfer. Using a sequence-independent approach to identify virus contigs, the team created a virus-like particle (VLP) database of 1,104 contigs. Following induction of colitis, the phageome composition changed, resulting in a decrease in contig abundances and diversity after 42 days. Mapping VLP reads showed that phages of the Caudovirales families and those that infect Enterobacteriaceae and Enterococci were enriched in mice with colitis at day 42. Abundance of some phages correlated with that of their host bacteria, indicating a link of phages and pathobionts during IBD.

The team also mapped phage DNA sequencing reads from mice to phage contigs from healthy individuals and patients with IBD. Comparative analysis of contig read coverage from healthy individuals with those from healthy or colitic mice showed an overlap of 25%. Fewer contigs from healthy humans were mapped to those from mice with colitis and some mapped to healthy animals only. Contigs from patients with IBD had a 33% overlap with reads from healthy or colitic mice. Contig read coverage was better in mice with colitis than in healthy animals and some matched with those from diseased mice only. These data indicate that mouse colitis models can be useful tools to study phage–bacterial interactions.

Clemens Thoma

ORIGINAL ARTICLE Duerkop, B. A. et al. Murine colitis reveals a disease-associated bacteriophage community. *Nat. Microbiol.* https://doi.org/10.1038/s41564-018-0210-y (2018)

PANCREATIC CANCER

Balance of stresses guides metastasis

A new study shows that unresolved endoplasmic reticulum stress (ERS) in pancreatic quiescent disseminated cancer cells (DCCs) enables these cells to escape killing mediated by adaptive T cells. These DCCs seem to function as latent metastases that can form macrometastases in the absence of T cells when ERS is resolved. The study provides a model that explains rapid metastasis in patients who have undergone surgical resection of their primary pancreatic tumour.

DCCs have been found in the context of different cancers, but the reason for their quiescence is not fully understood. Arnaud Pommier, first author of the study, and colleagues have now evaluated the role of DCCs in pancreatic cancer. Microscopy of tumour and liver samples from patients without clinically detectable metastases revealed single DCCs in the liver that were negative for cytokeratin 19 (CK19), Ki67 and major histocompatibility complex class I (MHCI) expression. By contrast, cancer cells from growing metastases were positive for these markers.

"Previous work identified an efficient endogenous adaptive immune response against pancreatic cancer," explains Pommier. "Hence, we thought that pancreatic cancer metastasis probably occurs in the context of an ongoing adaptive immune response and developed a metastasis model in this setting." Using genetically modified pancreatic cancer cells that could be selectively eliminated, the team established subcutaneous tumours in mice for 2 weeks to enable development of a T cell response. Following tumour elimination, these pre-immunized mice as well as naive mice were challenged with the same tumour cells injected into the spleen to mimic liver seeding. In naive mice, tumour cells established hepatic metastasis, whereas no metastatic foci were observed in immunized mice after day 5; however, depletion of $CD4^+$ or $CD8^+$ T cells enabled metastases development. Notably, the livers of immunized mice contained DCCs characterized by the absence of CK19, E-cadherin (E-cad), and MHCI expression as well as proliferation markers, recapitulating the findings in human samples. T cell depletion after 3 weeks or 9 weeks resulted in macrometastases, albeit in fewer mice in the 9-week group, possibly owing to the presence of fewer DCCs caused by spontaneous re-expression of MHCI and T cell killing over time.

Examining DCC origin, the team found a subpopulation of nonproliferating pancreatic cancer cells in their cell line that were $E\text{-cad}^-$, $CK19^-$ and $MHCI^-$. When $E\text{-cad}^+$ cells were injected into immunized mice, no macrometastases developed and DCCs were absent, whereas DCCs were present when $E\text{-cad}^-$ cells were injected. These results suggest that two phenotypic subtypes exist that have developmental plasticity controlled by a cell-autonomous switch, and that quiescent $E\text{-cad}^-$ cells are precursors to proliferating $E\text{-cad}^+$ cells.

The team then investigated cellular processes that control the phenotypic divergence. RNA sequencing revealed that ERS response and cell division were the most upregulated and downregulated pathways, respectively, in $E\text{-cad}^-$ cells compared with $E\text{-cad}^+$ cells. *Ddit3*, which encodes a transcription factor of the ERS response, was the most upregulated mRNA in $E\text{-cad}^-$ cells and was expressed in DCCs, but not in macrometastases. Examining ERS pathways, the team found activation of the unfolded protein response regardless of E-cad expression; however, in $E\text{-cad}^-$ cells, activation of the inositol-requiring enzyme 1 (IRE1a) pathway, which is required for ERS recovery, was lacking. Treatment of DCCs with an agent to relieve ERS resulted in increased MHCI expression and proliferation in vitro, and development of $MHCI^+$ macrometastases or reduced numbers of DCCs in immunized mice with or without T cell depletion, respectively. Further experiments showed that induction of the IRE1a downstream effector XBP1s, which promotes protein folding in the endoplasmic reticulum, resulted in responses similar to those seen with the ERS-relieving agent.

"Our results identify a new mechanism of immune-mediated latency in which T cells actively target proliferating cells but are inefficient against latent cells; thus, disruption of the T cell response would be necessary for growth of metastases," summarizes Pommier. "In patients with pancreatic cancer, surgery alone might be sufficient to disrupt the T cell response and enable latent cancer cells to grow into overt metastases. Going forward, we are evaluating implications for metastatic relapse and hope to set up a new standard of care for those undergoing pancreatectomy by managing glucocorticoid levels perioperatively."

Clemens Thoma

ORIGINAL ARTICLE Pommier, A. et al. Unresolved endoplasmic reticulum stress engenders immune-resistant, latent pancreatic cancer metastases. *Science* **360**, eaao4908 (2018)