TUMOUR MICROENVIRONMENT

Fungi complements cancer

Establishing associations between bacterial dysbiosis and cancer onset and progression as well as patient outcomes has been the primary focus of studies of the cancer microbiome to date. Yet, microbiota extends beyond simply bacteria. Taking this into consideration, the groups of Deepak Saxena and George Miller at New York University have now linked alterations in the fungal component (mycobiome) of the pancreatic microbiome, which activate the complement cascade, to pancreatic ductal adenocarcinoma (PDAC) growth and progression.

To assess whether intrapancreatic fungal content changed during tumorigenesis, the authors examined human PDAC specimens and a mouse model of slowly progressive PDAC generated by expressing oncogenic KRAS in pancreatic progenitor cells (*Ptf1a^{cre};LSL-Kras^{G12D}* mice; shortened to KC mice herein). An increased abundance of fungi was found in tumours from both patients with PDAC and KC mice relative to normal pancreatic tissue of healthy individuals and wild-type mice, respectively, raising the question of how pancreatic fungal infiltration might arise. The gut and pancreatic duct are directly linked by the sphincter of Oddi, which suggested to the researchers that translocation of the gut mycobiota could occur via this route. Testing their hypothesis by administering fluorescently tagged *Saccharomyces cerevisiae* to wild-type and KC mice via oral gavage demonstrated that within 30 min labelled fungi could be found in the pancreas.

Next, fungal DNA was extracted from gut and pancreatic tissues of KC mice and sequenced. This revealed that the gut mycobiome did not substantially overlap with that of the tumour mycobiome. Specific compositional differences included reduced taxonomic diversity as well as dominance of one particular genus, *Malassezia*, in pancreatic tumour tissues

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compared with the gut. Importantly, identical observations were made by sequencing tumour tissue and faecal samples (as an indicator of the gut mycobiome) from patients with PDAC, validating the mouse data.

To establish whether fungal dysbiosis could in turn drive the progression of PDAC, the mycobiome was ablated by feeding KC mice an antifungal agent known as amphotericin B. Treatment with the antifungal drug could protect against the progression of pancreatic dysplasia. Furthermore, ablation of the mycobiome in mice bearing orthotopic aggressive PDAC tumours (derived from tumour cells of the *Pdx1^{cre};Kras^{G12D};Trp53^{R172H}* (KPC) mouse model) could reduce tumour burden as well as enhance the effect of the chemotherapy gemcitabine.

The authors then performed repopulation experiments in which specific fungal species were adoptively transferred into amphotericin B-treated mice orthotopically implanted with PDAC tumour cells. Only *Malassezia globosa* and not *Candida* sp., *Saccharomyces cerevisiae* or *Aspergillus* sp. could accelerate tumour growth. This suggested that *M. globosa*, which was enriched in both human PDAC tumours and KC mouse tumours, was sufficient to advance PDAC progression. However, it remains to be determined whether imbalances of the pancreatic mycobiome

are a cause or consequence of tumorigenesis.

To rule out benign pancreatic inflammation as a trigger for perturbations in the pancreatic mycobiome, experimental pancreatitis was induced in wild-type mice. This did not result in an expansion of fungi within the pancreas and administration of antifungal agents had no effect on the phenotype, in line with fungal dysbiosis being specifically associated with tumorigenesis.

The innate immune system can recognize sugar motifs in fungal walls through engagement with the opsonin mannose-binding lectin (MBL), which consequently triggers the lectin pathway of the complement cascade. As the complement system has previously been implicated in promoting cancer, the authors sought to investigate a potential role for MBL in pancreatic cancer. In support of this, the progression of pancreatic dysplasia was hindered in MBL-null mice carrying orthotopic PDAC tumours. Moreover, under these conditions fungal ablation could not provide protection against tumour growth, and recolonization with *M. globosa* was ineffective at enhancing tumour growth.

Downstream of MBL in the complement cascade, the central component C3 is cleaved into the bioactive fragments C3a and C3b. Accordingly, *C3^{-/-}* mice implanted with orthotopic PDAC tumours displayed reduced tumour growth, which could not be further decreased with fungal ablation. Collectively, these experiments point towards fungi being able to functionally facilitate PDAC progression via MBL–C3 pathway activation.

Given that bacterial dysbiosis has already been reported to influence PDAC progression, it will be interesting for future research to determine the relationships between the various microbial communities during tumorigenesis and the potential effect that therapeutic targeting of one community might have on the other.

Anna Dart

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