EPITHELIAL CARCINOGENESIS GROUP

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OVERVIEW

We focus on the molecular pathophysiology of pancreatic ductal adenocarcinoma (PDAC) and urothelial bladder carcinoma (UBC) taking a disease-oriented approach. These tumours present very distinct clinical challenges. We learn from patient samples, cultured cells/organoids, and genetically modified mice. To translate the findings, we bring this knowledge to a "population" level leveraging on information and samples from large patient cohorts together with Núria Malats (CNIO).

"We have found that antibiotic administration and gut flora depletion rescues a genetic defect present in *Nr5a2* heterozygous mice that sensitises them to acute pancreatic damage and to PDAC."

PDAC has a dismal prognosis even when diagnosed early. We aim to dissect the molecular mechanisms involved in very early steps of tumour development, harnessing the excellent genetic mouse models available. A main hypothesis is that cell differentiation is an early and potent tumour suppressor mechanism. Understanding the contribution of early molecular events is crucial to design better strategies for prevention and early tumour detection.

UBC presents with very wide clinical and pathological heterogeneity. We aim to acquire knowledge about the underlying molecular pathways and to apply it for improved prediction of outcome and therapy.

RESEARCH HIGHLIGHTS

Pancreatic cancer molecular pathophysiology

In recent years, GWAS have identified a variety of common genetic variants associated with PDAC risk. Several of them are associated with genes involved in acinar cell biology, including *NR5A2* and *HNF1A*, coding for transcription factors required for full acinar differentiation that we have extensively studied. A few other GWAS hits associate with genes involved in acinar function, such as *XBP1* and *CTRB1/2*. These observations have strengthened the notion, pioneered by our lab, that cell differentiation is the first tumour suppressor mechanism in the pancreas. Among the processes participating therein are inflammation and the ER stress response. *Nr5a2* heterozygous mice display more damage and are not able to recover properly upon induction of a mild acute pancreatitis. In addition, they are more susceptible to mutant *KRas*-driven PDAC. Among the modifiable factors that may cooperate with this genetic defect to drive PDAC, we tested diet and the gut microbiome. A high fat diet does not add to the pancreatitis phenotype of *Nr5a2* heterozygous mice. In contrast, antibiotic administration and depletion of the gut microbiota rescues the genetic defect observed upon pancreatitis induction. 16S rDNA analysis does not reveal major differences in the faecal microbiome of wild type and *Nr5a2* heterozygous mice. A variety of experiments fail to support the contribution of heterozygosity at the intestinal level. Transcriptomic changes analysis of the pancreas reveals significant changes both in basal conditions and during pancreatitis. Most notably, in mice that received antibiotics we find an up-regulation of the acinar programme and of mitochondrial pathways and a down-regulation of cell cycle and inflammatory pathways. *Nr5a2* heterozygous mice have higher levels of CD4+ cells in blood, and antibiotic administration reduces their CD4+ cell levels. Gut microbiome reconstitution results in increased CD4+ cell counts (FIGURE 1). Our observations suggest that the gut microbiome induces a basal inflammatory state that contributes to disease, the modulation of which could be exploited therapeutically.

Urothelial bladder carcinoma (UBC) genetics, biology, and clinical translation

We focus on understanding 2 new UBC tumour suppressor genes that we identified through exome sequencing: *STAG2* and *RBM10*. *STAG2* codes for a cohesin subunit, and *RBM10* codes for a splicing regulator. We have generated conditional mouse models for these 2 genes and are exploring their role in development and urothelial biology, as well as their cooperation with other cancer genes.

Increasing evidence shows that STAG2 acts as a tumour suppressor through rather unique mechanisms, largely unrelated to the canonical role of cohesin in chromosome segregation. *STAG2* alterations occur early during tumourigenesis. Therefore, we are using both normal urothelial cells and tumour cell lines to identify the impact of STAG2 at the genomic and cellular levels. Using RT112 cells, we have integrated ChIP-Seq, HiC chromatin interaction data, and RNA-Seq to assess the impact of STAG2 knockdown. The cohesin-STAG2 complex mediates short- and mid-range interactions that engage genes at higher frequency than cohesin-STAG1. STAG2 knockdown results in the down-regulation of luminal differentiation programmes and up-regulation of basal programmes. These findings are at odds with the fact that *STAG2* mutations are associated with luminal-type bladder cancers, suggesting an intermediate luminal differentiation phenotype. STAG2 knockdown does not affect compartment and domain boundaries, but it rewires intra-TAD DNA interactions and leads to the de-repression of lineage specifying genes (in collaboration with M. Martí-Renom, *CRG*, Barcelona).

Our translational studies expand several clinical trials with a strong translational component carried out in collaboration with Núria Malats and Spanish uro-oncologists. ■

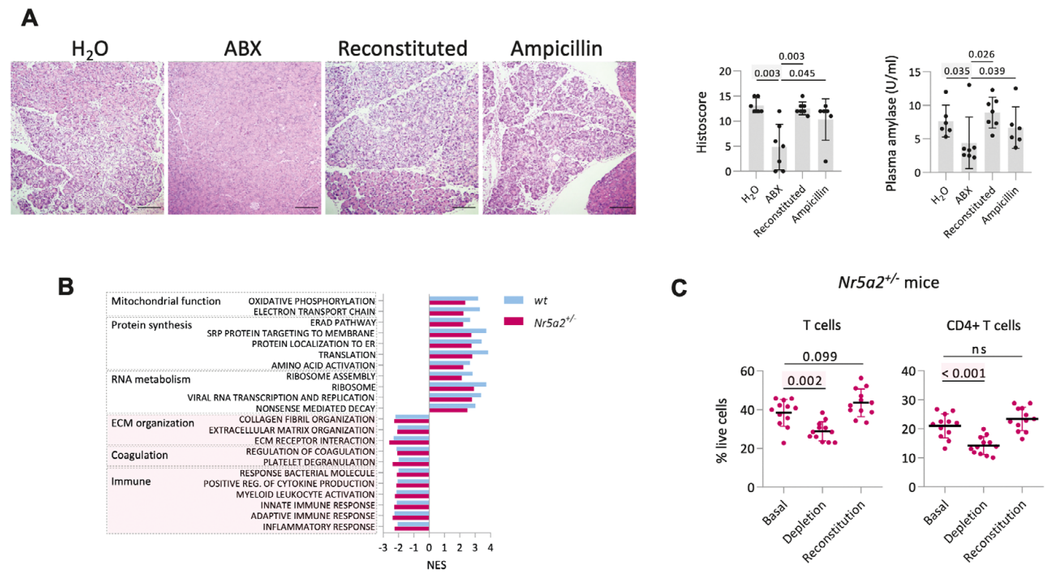

FIGURE 1 Antibiotic-mediated gut flora depletion rescues the genetic defect of *Nr5a2*$^{+/-}$ mice, as shown at the histological level upon induction of acute pancreatitis (**A**). In basal conditions, antibiotic administration has anti-inflammatory effects in the pancreas as shown by RNA-Seq pathway analysis (**B**). Antibiotic administration induces a significant decrease of CD4+ cells in the blood that is reversed upon gut flora reconstitution by co-housing (**C**).

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