EPITHELIAL CARCINOGENESIS GROUP

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Titulado Superior (Advanced Degree)

OVERVIEW

We focus on the molecular pathophysiology of pancreatic ductal adenocarcinoma (PDAC) and urothelial carcinoma (UC), with a disease-oriented approach. We use patient samples, cultured cells, and genetically modified mice, giving a similar weight to the 3 model systems. Observations made at either of these levels are then extended through additional work. To translate the findings, we bring this knowledge to a 'population' level leveraging on information and samples from large patient cohorts.

"We have shown that, in the pancreas, the control of cell differentiation and the suppression of inflammation depend on similar transcriptional regulators indicating that both processes are tightly linked."

In PDAC, a main hypothesis is that cell differentiation is a potent tumour suppressor mechanism acting early in carcinogenesis. We use the excellent genetic mouse models available because these processes cannot be readily studied in humans. In mice, PDAC can originate in pancreatic progenitors and in adult acinar and ductal cells. Understanding the contribution of early molecular events is crucial to design better strategies for early tumour detection and prevention in subjects at risk.

In UC, we focus on identifying new genes, using them for improved tumour taxonomy, characterising the mechanisms of action, and applying this knowledge for improved prediction of outcome and therapy.

RESEARCH HIGHLIGHTS

Pancreas cancer molecular pathophysiology

The genetic/genomic changes associated with PDAC have been extensively described over the last few years by the genome consortia, but the contribution of precursor lesions and the molecular changes that precede tumour development are less well established. Our lab has pioneered the notion that incomplete acinar cell differentiation is associated with a scenario of pre-inflammation or inflammation and with predisposition to PDAC development using mutant *KRas*-driven genetic mouse models. These studies provide the basis for the pharmacological – or genetic - manipulation of acinar differentiation as a tumour preventative strategy.

NR5A2 is an orphan nuclear receptor for which putative endogenous ligands as well as pharmacological agonists have recently been identified. In mice, *Nr5a2* germline heterozygosity is associated with a pre-inflammatory state that sensitises the mice to the oncogenic effects of mutant *KRas*. Deletion of one *Nr5a2* allele is sufficient to cause a striking genomic redistribution of the protein in cooperation with AP-1 components. To further explore how this occurs, we have analysed the NR5A2 interactome using immunoprecipitation and mass-spectrometry. We find that reduction of NR5A2 protein levels by 50% (either genetically or during pancreatitis) is also associated with profound effects on the interactome, highlighting the relevance of subtle changes in protein dosage in cells; one of the proteins identified is the ubiquitous transcription factor NFIC (FIGURE 1A,B). At the transcriptomic level, *Nfic*$^{-/-}$ pancreata display a mild defect in acinar cell maturation as well as a significant down-regulation of the protein synthesis machinery. NFIC is a novel regulator of acinar differentiation playing an important role in the endoplasmic reticulum stress response. Similar to knockouts of other genes coding for proteins involved in acinar homeostasis, constitutive *Nfic*-null mice developed significantly more PanINs in a mutant KRAS context. The function of NFIC in acinar cells appears to be highly conserved between mice and humans (FIGURE 1C).

Urothelial carcinoma (UC) genetics, biology, and clinical translation

We are interested in refining our understanding of new genes involved in UC, using organoids to unravel their function, and to apply this knowledge in the clinical setting.

Through exome sequencing we identified mutations in *STAG2*, coding for a cohesin subunit, and in *RBM10*, coding for a splicing regulator, as new UC genes that are more broadly involved in human cancer. We have generated conditional mouse models for these two genes and are exploring their role in development and urothelial biology as well as their cooperation with other bladder cancer genes.

RBM10 somatic mutations occur in several epithelial tumour types, including UC. Germline *RBM10* mutations are associated with TARP syndrome. Our preliminary studies indicate that *Rbm10*-null mice recapitulate facets of this developmental condition. We have generated *Rbm10*-null normal urothelial organoids and are characterising their biological features. In addition, we collaborate with J. Paramio (*CIEMAT*, Madrid) to identify how tumour cells bypass growth requirements in organoid cultures. Also, through single-cell RNA-Seq, we are identifying urothelial cell populations that could shed light on the cell of origin of UC.

In collaboration with J. Valcárcel (*CRG*, Barcelona), we are analysing the mechanisms through which *RBM10* contributes to UC development using a combination of cellular, molecular and bioinformatics approaches. In addition, we are assessing whether RBM10-mutant tumours display specific therapeutic vulnerabilities using a variety of experimental strategies.

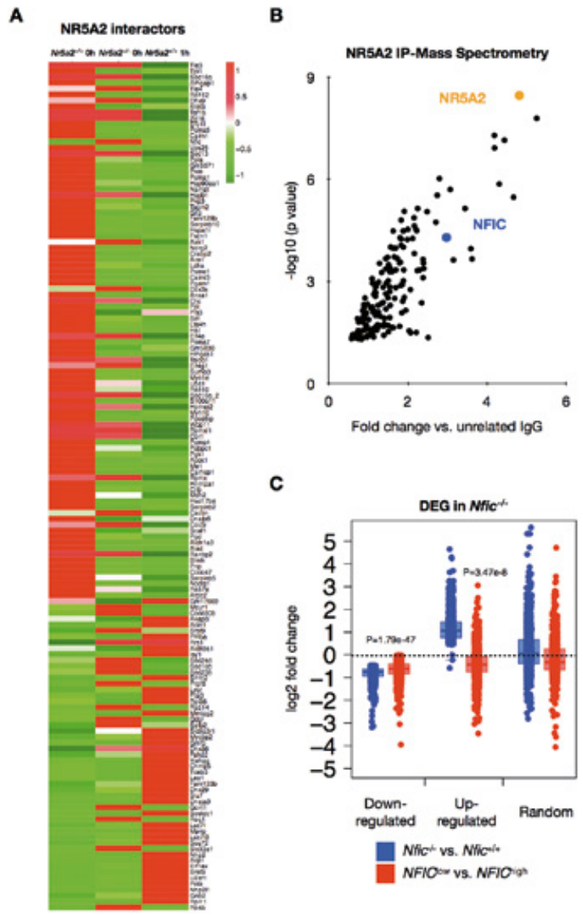

Figure 1 The NR5A2 interactome is dynamic and reveals novel players involved in acinar homeostasis. (**A**) NR5A2 interactors identified by immunoprecipitation with anti-NR5A2 antibodies and mass spectrometry using pancreatic tissue from wild type mice in basal conditions (*Nr5a2*$^{+/+}$ 0h), or 1 h after administration of one dose of caerulein (*Nr5a2*$^{+/+}$ 1h), and from *Nr5a2* heterozygous mice in basal conditions (*Nr5a2*$^{+/-}$ 0h). (**B**) NFIC is a novel NR5A2 interactor identified in all three analysed settings. (**C**) Genesets including differentially expressed genes (DEG) in the pancreas of young *Nfic*$^{-/-}$ vs. wild type mice: down-regulated genes are expressed at significantly lower levels in normal pancreas from subjects with low levels of *NFIC* (comparison of lower vs top decile of *NFIC* mRNA expression) (in collaboration with F. García and J. Muñoz, CNIO Proteomics Unit).

Our studies with patient samples have provided novel markers predictive of response to cisplatin-based chemotherapy and are guiding the design of novel clinical trials with targeted therapies and immune checkpoint inhibitors in collaboration with N. Malats, A. Font, D. Castellano, and an extended group of Spanish uro-oncologists. ■

PUBLICATIONS

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AWARDS AND RECOGNITION

- "Constantes y Vitales" Award for the best publication in Biomedicine 2018, *Atresmedia/La Sexta*, Spain.