

Epithelial Carcinogenesis *Group*

Summary

Our Group is interested in understanding the molecular/cellular mechanisms involved in pancreatic and bladder cancer and applying such knowledge at the clinical level. We adopt a transversal approach and aim to address important questions through the biochemical or genetic analysis of genes/molecules involved in these tumours and later test the relevance of the findings in the clinical setting – often using mouse models as an intermediate step.

Alternatively, we make observations in molecular pathology/epidemiology studies and then test the mechanisms involved using *in vitro* and *in vivo* experimental systems.

Strategic Goals

Pancreatic cancer:

- Understand how changes in cell differentiation contribute to the development of preneoplastic and neoplastic lesions
- Establish new animal models of pancreatic cancer focusing on the early events involved in tumour development and the role of the stroma

Bladder cancer:

- Identify the genes/pathways involved in the development of bladder cancer subtypes and the mechanisms involved therein
- Establish molecular markers of recurrence/progression and response to therapy
- Develop an integrative 'omics' approach to understanding tumour development/progression

Francisco X. Real *Group Leader*



Paco Real was born in Barcelona in 1957 and obtained his MD (1980) and PhD (1986) from the *Universitat Autònoma de Barcelona*. He trained in Medical Oncology at the Memorial Sloan-Kettering Cancer Center in New York (USA), where he was appointed Research Associate in 1985 and Staff Physician for the Medical Oncology Division in 1986.

His work focused on the development of monoclonal antibody-based strategies for the treatment of melanoma and epithelial cancers. Alongside J. Mendelsohn, he led the first clinical trial with anti-EGFR monoclonal antibodies in humans.

In 1988 he joined the *Institut Municipal d'Investigació Mèdica* in Barcelona, where he was Coordinator of the Cell and Molecular Biology Unit until 2007. In 1998, he joined the staff of the *Universitat Pompeu Fabra* (UPF), Barcelona, where he has been Professor of Cell Biology since 2004. He joined the CNIO in September 2007 as Senior Group Leader.

Paco has made important contributions in the cell/molecular biology of pancreatic and bladder cancer. He has served on the Advisory Editorial Boards for the *Biochemical J* (1994 – 2008) and the Council of the European Pancreatic Club (2005 – 2008). He has served as President of the Spanish Association for Cancer Research (ASEICA) (2002 – 2004) and received several awards including the Charles Mérieux Chair at the *Ecole Normale Supérieure* in Lyon, France (1997).



Post-doctoral fellows: Monserrat Blanco (until October), Elena Cibrián (until August), Julie Earl, Miriam Marqués (since April), Paola Martinelli, Marinela Méndez, Elena Ortiz.
Graduate students: Jaroslaw Cendrowski, M. Luisa Morais (until April), Laia Richart, Andreia A. Vaqueirinho. **Technicians:** Ariel E. Casanova, Natalia Del Pozo, Daniel Pastor.

Highlights

Pancreatic cancer molecular pathophysiology

Our Group is interested in understanding how changes in cell differentiation contribute to malignant transformation in the pancreas, including the role of acinar cells and ductal preneoplastic lesions. There is increasing evidence that pancreatic ductal adenocarcinoma (PDAC) can originate in both ductal and acinar cells. The elucidation of the contribution of these cell types in PDAC progression is crucial to better design strategies for early tumour detection in high risk subjects.

Cell differentiation and pancreatic diseases

Using *in vitro* cultures of mouse exocrine pancreas we have shown that acinar cells can acquire progenitor features – including the assembly of an embryonic PTF1 transcriptional complex bound to its corresponding promoters and the expression of progenitor markers (Figure 1). These cells do not proliferate and rather activate a senescence-like programme involving up-regulation of p53, p21, p16, and Dec1. Importantly these markers are also found to be up-regulated in ductular complexes found in rats and mice after pancreatic duct ligation. We are now extending these findings to human samples to assess whether such barriers can contribute to tumour suppression.

Using different approaches we have identified new genes involved in the regulation of acinar cell differentiation, including *Hnf1α* and the Inhibitor of β -Catenin, *ICAT*. In collaboration with X. Molero at the *Hospital Vall d'Hebron*, Barcelona, we have shown that *Hnf1α* inactivation leads to a defect in digestive enzyme production and increased acinar cell proliferation, as well as an altered recovery from acute cerulein-induced pancreatitis. *ICAT* binds Ptf1a and modulates the function of the PTF1 complex. Using genetic mouse models we plan to explore whether *Hnf1α* haploinsufficiency and *ICAT* overexpression modulate the development and/or progression of PDAC.

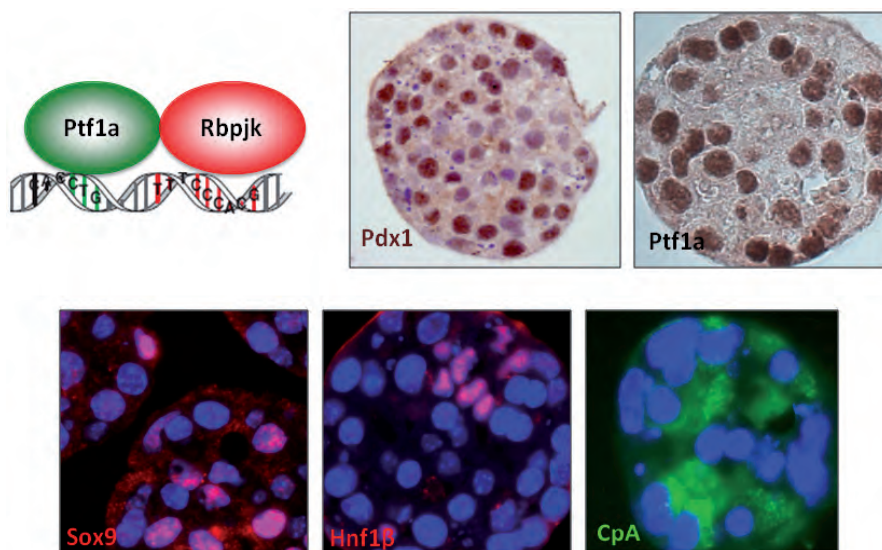


Figure 1: Murine acinar cells acquire *in vitro* features of pancreatic progenitors. Their phenotype is characterised by co-expression of Pdx1 and Ptf1a - as well as Hes1, Sox9, Hnf1 β , and low levels of carboxypeptidase A (Cpa) - and the presence of the embryonic PTF1 complex (containing Rbpjk rather than Rbpjl).

Growth control mechanisms still active in pancreatic ductal adenocarcinoma

Over the last few years we have characterised a small number of PDAC lines which maintain contact inhibition despite *KRAS* mutations and *p16*, *TP53*, and *SMAD4* inactivation. This striking feature reveals that the preservation of growth control mechanisms may offer therapeutic potential. We have identified transcriptional networks that are regulated in these cells during contact inhibition and are focusing on *GATA6*, an oncogene that is amplified in 10% of PDAC.

Bladder cancer molecular genetics

Our efforts focus on understanding the role of mutations in *FGFR3* and in genes involved in the phosphoinositide-3-kinase (PI3K) pathway in urothelial

cell carcinoma (UCC). One major goal is to establish how these mutations may determine patient outcome, response to therapy, and prediction of response to targeted therapy. *FGFR3* mutation and transcript overexpression *in vitro* are strong predictors of sensitivity to FGFR chemical inhibitors.

We have also defined a crosstalk between *FGFR3* and *FGFR1* which is involved in the regulation of the epithelial phenotype in UCC. Regarding the PI3K pathway, *PIK3CA* mutations are effective *in vitro* predictors of the response to selective inhibitors, whereas drugs that also inhibit mTOR have a broader effect.

The skin as a model for urothelial cell carcinoma

We have extensive evidence that shows low grade papillary UCC shares many genetic alterations with seborrheic keratosis (SK), a benign skin neoplasm with no malignant potential. We have prospectively collected a large panel of SK samples with multiple lesions from each individual and have found that they commonly harbour point mutations in the oncogenes involved in papillary UCC, such as *FGFR3* (70%), *PIK3CA* (50%), *HRAS*, and *KRAS*. These lesions are generally clonal and commonly contain more than one oncogenic mutation but they do not activate a senescence programme, supporting the existence of alternative mechanisms that limit tumour progression.

The mutational analysis – with X chromosome inactivation studies – suggests that these noticeably independent lesions likely share a common origin (Figure 2). These studies shed light on the mechanisms that may operate in other tissues where such analyses are more difficult to perform.

This work was carried out in collaboration with R. Pujol's Group, *Institut Municipal d'Investigació Mèdica (IMIM-Hospital del Mar)*, Barcelona, and the Group of C. Hafner, *Universität Regensburg*, Germany.

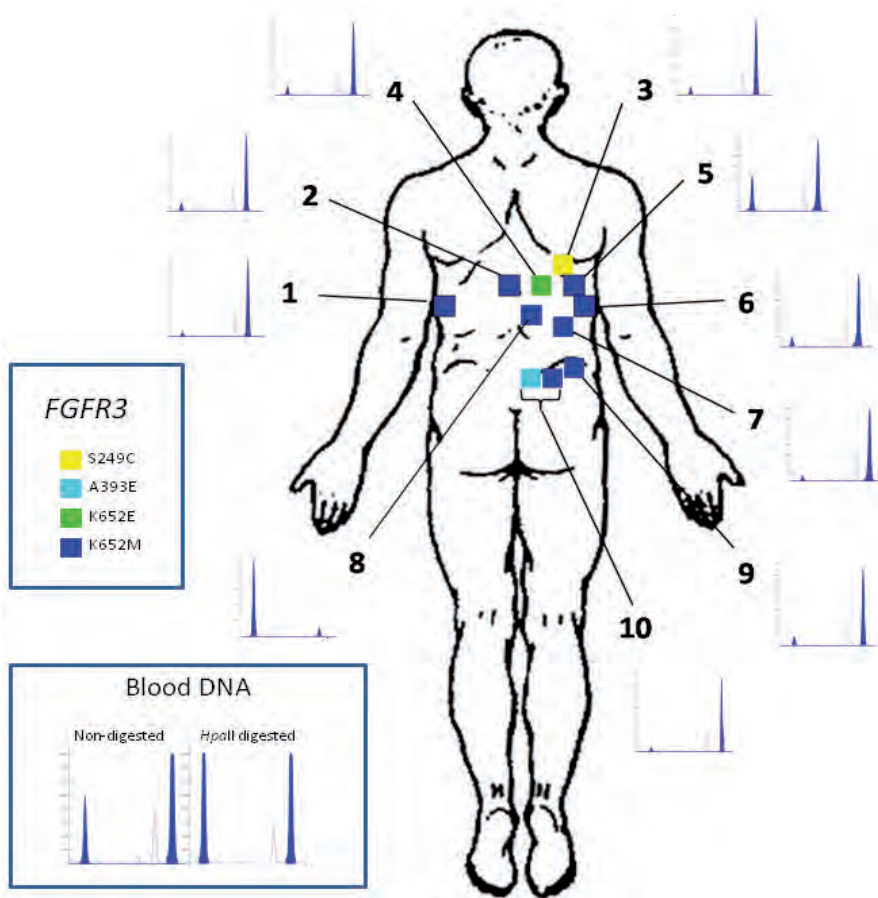


Figure 2: *FGFR3* in seborrheic keratosis reveals a clustering of lesions harbouring the K652M mutation. The relatedness of noticeable independent lesions is shown using HUMARA. This assay allows the studying of the X chromosome inactivation pattern in women who are heterozygous for a methylation-sensitive restriction site at the Human Androgen Receptor locus.

Gene copy number variation (CNV) and UCC

We are focusing on the role of germline and somatic gene CNVs in bladder cancer. We have compared leukocyte and tumour DNA from the same patient with the Illumina Infinium array. The initial analysis of leukocyte DNA reveals a wide range of structural variations found in both, controls and patients with UCC, pointing to their potential contribution to bladder cancer susceptibility and progression. The findings also reveal the evolving nature of the human genome at the individual level and the relevance

of these studies as markers of disease susceptibility.

This work was carried out in collaboration with Núria Malats and Alfonso Valencia's Groups at the CNIO, L. Pérez-Jurado, *Universitat Pompeu Fabra*, Barcelona, and S. Chanock, Core Genotyping Facility, NCI, Bethesda (USA).

Publications

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