

Epithelial Carcinogenesis *Group*



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.

He joined the *Institut Municipal d'Investigació Mèdica* in Barcelona (1988) where he was Coordinator of the Cell and Molecular Biology Unit until 2007. In 1998 he joined the staff of *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 and currently chairs the CNIO Scientific Committee.

Paco has made important contributions in the cell/molecular biology of pancreatic and bladder cancer and, most recently, the genetic analysis of benign epidermal tumours. He has served on the Advisory Editorial Boards of *Biochemical Journal* (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).

Summary

The main interest of our Group is to understand the molecular/cellular mechanisms involved in pancreatic and bladder cancer by adopting a disease-oriented approach. Our strategy builds on a structure similar to a pyramid of an equilateral triangle base. The pyramid's 3 vertexes represent our working models: patient samples, cell cultures, and genetically modified mice – all three models are equally balanced.

The third dimension comes from the extension of this knowledge to "population" studies whereby we bring the biology to patient studies. We are primarily interested in the genetic susceptibility to cancer and in developing more sophisticated molecular tools to predict patient outcome or response to therapy. This process is highly modular and our primary observations can be made at either of these levels and then be extended to additional studies.

Strategic Goals

Pancreatic cancer

- To understand how the molecular mechanisms involved in cell differentiation contribute to the development of preneoplastic and neoplastic conditions
- Establish new animal models of pancreatic cancer focusing on the early events involved in tumour development and the role of pancreatitis and 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 understand tumour subphenotypes as well as tumour progression



Staff scientists: Marinela Méndez and Victor J. Sánchez-Arevalo. **Post-doctoral fellows:** Julie Earl, Marta Flández (since April), Miriam Marqués, Paola Martinelli and Elena Ortiz (until September). **Graduate students:** Cristina Balbas (since September), Jaroslaw Cendrowski, Laia Richart and Andreia A. Vaqueirinho. **Technicians:** Ariel E. Casanova, Natalia del Pozo, Carme T. Guerrero (since June), Javier Langa (since June) and Daniel Pastor (until May).

Highlights

Pancreatic cancer molecular pathophysiology

Our Group is interested in understanding how the mechanisms that control exocrine cell differentiation contribute to malignant

transformation in the pancreas, including the role of both acinar cells and ductal preneoplastic lesions. There is increasing evidence that experimental 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. Acinar cells can acquire progenitor features *in vitro* when cultured in suspension – these include the assembly of an embryonic PTF1 transcriptional complex bound to the appropriate promoters and the expression of progenitor markers. 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 in rats and mice after pancreatic duct ligation.

We have shown that p53 exerts two major effects on epithelial cells *in vitro*: in its absence, the arrest in proliferation is completely abrogated and acinar cells become immortalised; using genetic lineage tracing we have also shown that these cells undergo an epithelial-mesenchymal transition (Figure 1). These *in vitro* studies therefore reveal a crucial role for p53 in epithelial cell homeostasis which is not observed upon analysis of pancreatic tissue from p53-null mice.

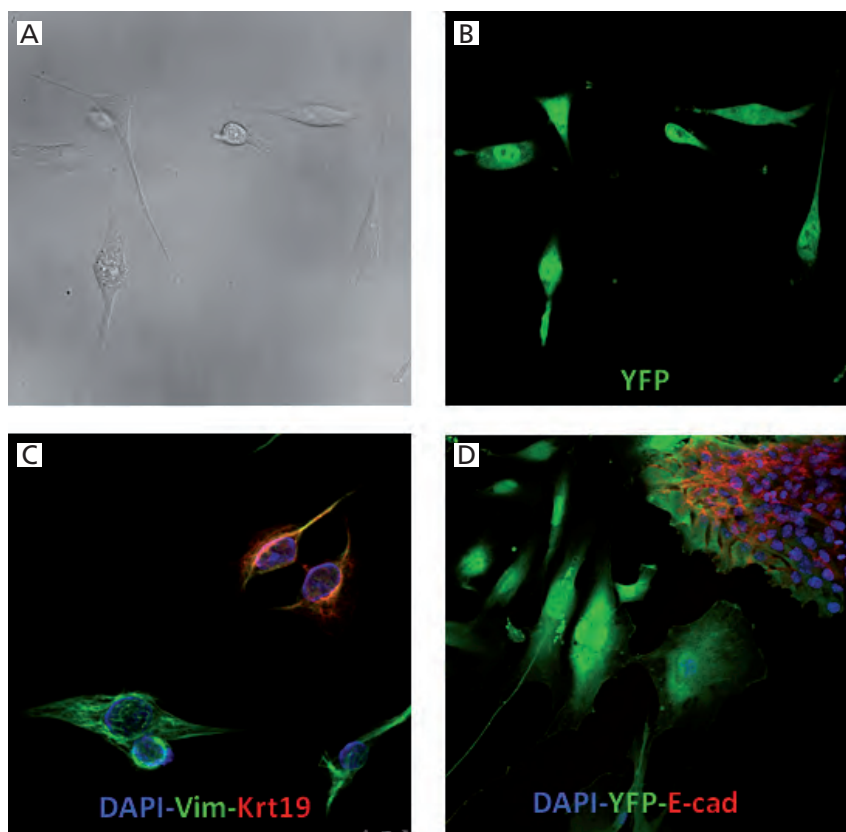


Figure 1: p53 controls both self-renewal and epithelial differentiation in normal mouse pancreatic cells. The exocrine pancreas was isolated from $Ptf1a^{Cre/+}; R26R-EYFP^{KI/+}; p53^{-/-}$ mice. After 12 passages, YFP-expressing cells of epithelial origin were isolated by sorting and analysed (A, B, C). Most cells lacked expression of epithelial markers yet were reliably labelled by YFP, indicating their epithelial origin. Cells expressing epithelial markers such as Krt19 or E-cadherin often co-expressed mesenchymal markers such as vimentin (C) and form compact colonies (D).

In addition, using both cultured cells and genetic mouse models, we are exploring the role of novel transcription factors and signalling molecules in pancreatic cell differentiation and malignant transformation.

Growth regulatory mechanisms still active in PDAC. We have characterised a small number of PDAC lines which maintain contact inhibition despite harbouring *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 several transcriptional networks putatively involved in this process and specifically focus on *GATA6*, *FOXA1/2*, *BPTF*, as well as *ETS1*.

This work on PDAC was carried out in close collaboration with the other groups also working on this tumour type at the CNIO (Mariano Barbacid from the Experimental Oncology Group; Christopher Heeschen from the Stem Cells and Cancer Group; Manuel Hidalgo from the Gastrointestinal Cancer Clinical Research Unit; and Núria Malats from the Genetic and Molecular Epidemiology Group).

Urothelial cell carcinoma (UCC) genetics

Understanding the role of *FGFR3* and *PIK3CA* as key oncogenes involved in UCC is a major focus in our laboratory. We have defined a cross talk between *FGFR3* and *FGFR1* which is involved in the regulation of the epithelial phenotype in UCC and may act as a critical switch in the control of tumour behaviour.

We are currently analysing the relevance of these findings *in vitro* as well as *in vivo* in UCC progression. To investigate how these oncogenes cooperate with tumour suppressors, we are using urothelial cultures derived from several mouse strains deficient in specific tumour suppressors.

Our work with seborrheic keratosis (SK), a benign epidermal lesion harbouring mutations in the same oncogenes that are commonly activated in UCC, is providing important clues about the mechanisms that protect epithelial cells from oncogenic stress: in SK, oncogene mutations fail to turn on a senescence response possibly due to the existence of negative regulatory feedback loops that prevent downstream pathway activation. We are developing culture methods for SK that will allow us to unravel the mechanisms that prevent oncogene-induced tumorigenic effects.

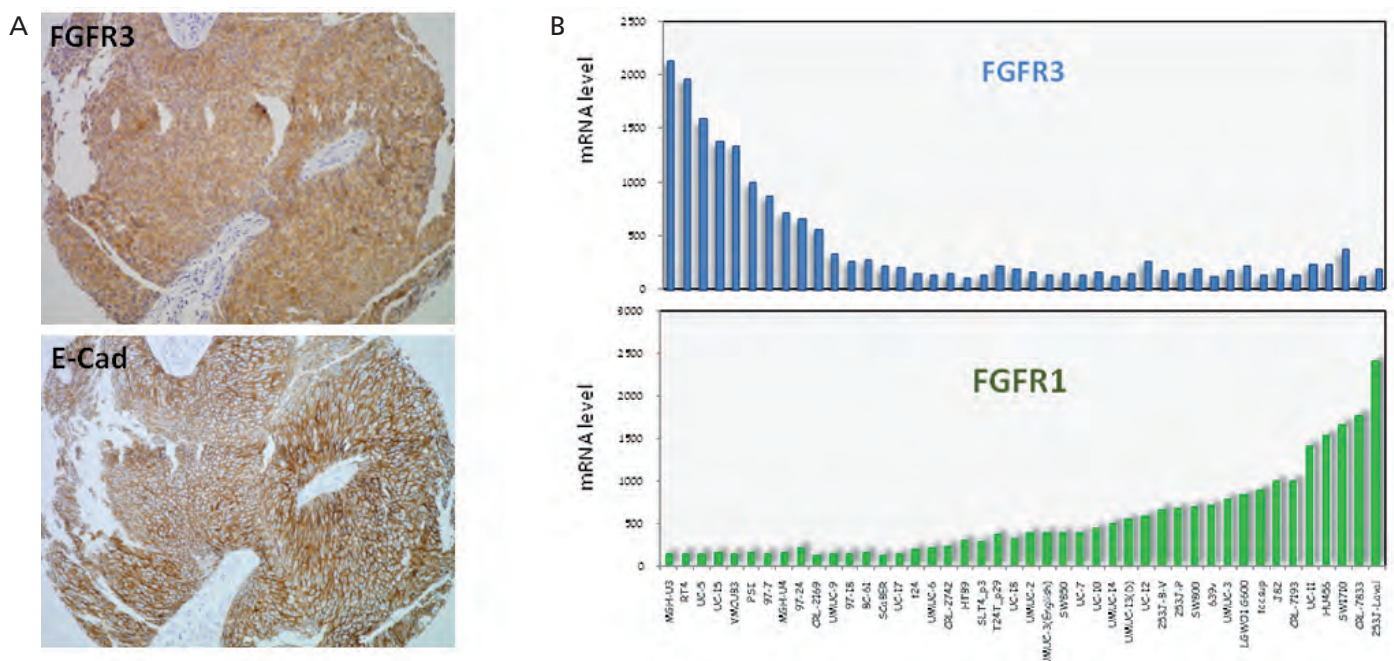


Figure 2: *FGFR3*, E-cadherin, *FGFR1* and bladder cancer phenotypes. *FGFR3* overexpression is strongly associated with papillary tumour growth and epithelial differentiation, including E-cadherin expression (A). *FGFR3* mRNA levels are inversely associated with *FGFR1* mRNA levels, as assessed by microarray analysis in a large panel of bladder cancer cell lines (B).

We are also beginning to implement studies on genomic changes using high resolution arrays with massive parallel sequencing, in order to analyse the mutational landscape of UCC.

The contribution of somatic embryonic mosaicism to adult cancer. Most current cancer models postulate that the genetic/genomic alterations associated with common cancers in the elderly occur during adult life. We have adopted a variety of strategies to demonstrate that somatic embryonic alterations (i.e. oncogene mutations and genomic aberrations) occur frequently, lead to mosaicism in adult tissues, and can contribute to disease, including cancer. Most notably, 1.7% of patients with UCC, as well as a similar proportion of control subjects, harbour aneuploidies and uniparental disomies in

peripheral blood leukocytes, which also occur in tumours of patients. We have also found that postzygotic mosaic activating mutations in oncogenes (i.e. *RAS*, *FGFR3*, *PIK3CA*) cause epidermal nevi and can contribute to UCC. These findings lead to a novel paradigm: embryonic mutations contribute to the genetic/genomic changes responsible for solid tumours in adults.

This work was carried out in close collaboration with Núria Malats (the Genetic and Molecular Epidemiology Group) and Alfonso Valencia (the Structural Computational Biology Group) at the CNIO, L. Pérez-Jurado, *Universitat Pompeu Fabra*, Barcelona, R. Pujol, *Hospital del Mar-IMIM*, Barcelona, C. Hafner, *Universität Regensburg* (Germany), and S. Chanock, the Core Genotyping Facility, NCI, Bethesda (USA).

Publications

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Awards and Recognition

Member, Scientific Advisory Board, Pancreatic Cancer Research, UK

Member, External Advisory Committee, *Instituto Ramón y Cajal de Investigación Sanitaria* (IRYCIS), Spain