

Experimental Oncology Group

Summary

We are continuing to take advantage of the power of mouse genetics to address fundamental questions such as the contribution of Ras pathways to cell proliferation, differentiation, and survival. We have also expanded the use of our K-Ras-driven lung and pancreas tumour models to identify synthetic lethal interactions with downstream targets that may unveil novel therapeutic strategies for clinical use. We are also characterising our mouse models for developmental NCFB syndromes to understand the consequences of constitutive, germline activation of the Ras mitogenic pathway.

Strategic Goals

- Genetically interrogate the contribution of Ras signalling pathways to normal homeostasis
- Develop mouse models for developmental syndromes caused by hyperactive Ras signalling pathways
- Develop mouse models for lung (NSCLC) and pancreatic (PDAC) tumours that recapitulate the natural history of human neoplasias
- Identify synthetic lethal interactions between K-Ras oncogenes and downstream effectors to unveil novel therapeutic strategies

Mariano Barbacid *Group Leader*

Mariano Barbacid was born in Madrid in 1949. He was awarded his PhD degree from the *Universidad Complutense de Madrid* in 1974. From 1974 – 1978 he trained as a Postdoctoral Fellow at the National Cancer Institute (NCI), Bethesda, Maryland, USA.

In 1978 he set up his own group to work on the molecular biology of human tumours. His work led to the isolation of the first human oncogene, H-Ras, in 1982. Other contributions of special relevance include the identification of Ras oncogenes as targets of chemical carcinogens (1984), the discovery of the Trk family of tyrosine kinase receptors as the signalling receptors for the NGF family of neurotrophic factors (1991) and the physiological role of the cell cycle Cdk5 (2003 – 2007).

He moved to the NCI campus in Frederick, Maryland (USA), as Head of the Developmental Oncology Section in 1984. In 1988, he joined the Bristol Myers-Squibb Pharmaceutical Research Institute in Princeton, New Jersey where he became Vice President of Oncology Drug Discovery. In 1998 he returned to Madrid to create and direct the CNIO (see Commentary in *Cell*, 129: 641-644, 2007).

Mariano Barbacid has authored 245 publications including 176 original articles and 24 invited reviews in refereed journals as well as 45 book chapters. The average impact factor of the 200 publications published in peer-reviewed journals is >12. His current Hirsch “h” factor is 86.

The relevance of his work has been recognised through several awards including the Young Investigator Award of the AACR (1986), the Steiner Prize (1988), the Ipsen Prize (1994), The *Jimenez Díaz* Award (2002), the Brupbacher Cancer Research Prize (2005), and the Medal of Honour of the International Agency for Research on Cancer (WHO) in 2007. He has been an EMBO Member since 1996.





Staff scientists: Matthias Drosten, Carmen Guerra, David Santamaría. **Post-doctoral fellows:** Chiara Ambrogio (since November), Sarah Francoz, Raquel García (since February). **Graduate students:** Andrea C. Aguilera (until November), Rafael B. Blasco, Antonio Cerqueira (until August), Miguel Ganuza, Isabel Hernández, Alberto Jiménez (until August), Sara Mainardi, Carolina Navas, Patricia Nieto (since September), Marta Puyol (until September), Jelena Urosevic (until October). **Technicians:** M. Carmen González, Marta San Román, Raquel Villar.

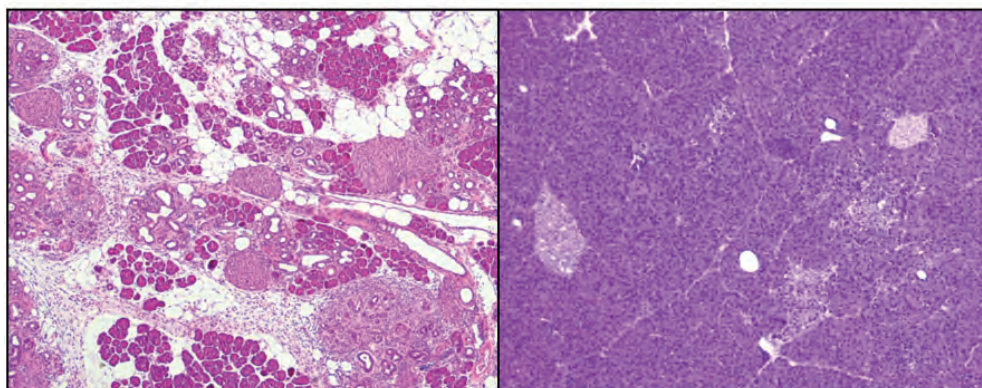
Highlights

Distinct contribution of the various Ras signalling pathways to cell proliferation, migration and survival: a genetic analysis

The Ras family of small GTPases, H-Ras, N-Ras and K-Ras are among some of the most studied proteins due to their central role in mediating mitogenic signalling and in human cancer. The molecular pathways involved in Ras signalling – primarily the Raf/Mek/Erk, PI3K/PTEN/Akt and RalGEF/Ral pathways – have been well defined. The precise contribution of each of these pathways to basic cellular functions has not however been analysed due to the inherent difficulty of selectively inhibiting each of these pathways.

To help solve this caveat we have generated Rasless MEFs carrying null H-Ras and N-Ras alleles along with a floxed K-Ras locus that can be ablated with an inducible Cre recombinase. We have used these MEFs to illustrate that Ras proteins are essential for cell proliferation and migration, but not for cell survival. These properties are unique to the Ras subfamily of proteins since other Ras-like small GTPases, even when constitutively active, could not compensate for the absence of Ras proteins. Only constitutive activation of components of the Raf/Mek/Erk pathway was sufficient to sustain normal proliferation and migration of MEFs devoid of Ras proteins. Activation of the PI3K/PTEN/Akt and RalGEF/Ral

K-Ras^{+/-LSLG12Vgeo};Elas-tTA/tetO-Cre mice



Control

Sulindac treatment

Figure 1: Sulindac treatment delays progression of PanIN lesions and PDAC. H&E staining of pancreata obtained from K-Ras^{+/-LSLG12Vgeo};Elas-tTA/tetO-Cre mice exposed to caerulein for three months untreated (left) or treated (right) with Sulindac for three additional months after cessation of the caerulein treatment.

pathways, either alone or in combination, failed to induce proliferation or migration of Rasless cells although they cooperated with Raf/Mek/Erk signalling to replicate the full response mediated by Ras signalling.

In contrast to current hypotheses, Ras signalling did not induce proliferation through the induction of D-type Cyclins. Rasless MEFs had normal levels of Cyclin D1 as well as Cyclin E. CyclinD1/Cdk4 and Cyclin E/Cdk2 complexes were also inactive. Direct inactivation of the pocket proteins or knock down of pRb relieved MEFs from their dependence on Ras signalling to proliferate.

Pancreatitis, inflammation and PDAC

We have previously described that expression of an endogenous K-Ras oncogene in adult pancreatic acinar cells does not result in tumour

development unless the mice suffer from mild chronic pancreatitis. These observations suggest that PDAC may stem from a combination of genetic (somatic K-Ras mutations) and non-genetic (tissue damage, inflammation) events. We have now demonstrated that even short term episodes of mild pancreatitis are sufficient to cooperate with K-Ras oncogenes in inducing the formation of PanINs that later progress to invasive PDAC.

To evaluate the contribution of inflammation on the development of PanINs and PDAC, we treated *K-Ras^{+/+}^{LSLG12V^{geo}};Elas-tTA/tetO-Cre* mice suffering from pancreatitis with Sulindac, a non-steroidal anti-inflammatory agent of the arylalkanoic acid class thought to inhibit COX-1 and COX-2 enzymes. Mice treated with Sulindac exhibited an extremely well preserved pancreas with limited parenchyma atrophy, few areas of inflammation and a limited number of PanINs (Figure 1). In contrast, mice with pancreatitis not exposed to Sulindac displayed multiple diffuse lesions, ranging from benign PanINs to invasive PDA with an abundance of high grade PanIN3 (Figure 1). Thus, induction of an inflammation response appears to be a key mechanism by which pancreatitis cooperates with K-Ras oncogenes to induce PDAC in adult mice.

Synthetic lethal interaction between K-Ras oncogenes and Cdk4 unveils a therapeutic strategy for non-small cell lung carcinoma

We have unveiled a synthetic lethal interaction between K-Ras oncogenes and Cdk4 in a mouse tumour model that closely recapitulates human NSCLC. Ablation of Cdk4, but not Cdk2 or Cdk6, induces an immediate senescence response only in lung cells expressing an endogenous K-Ras oncogene (Figure 2). No such response occurs in other K-Ras expressing tissues or in lung tissue expressing a single *Cdk4* allele. More importantly, targeting *Cdk4* alleles in advanced tumours detectable by computed tomography scanning also induced senescence and prevented tumour progression (Figure 3). These observations suggest that robust

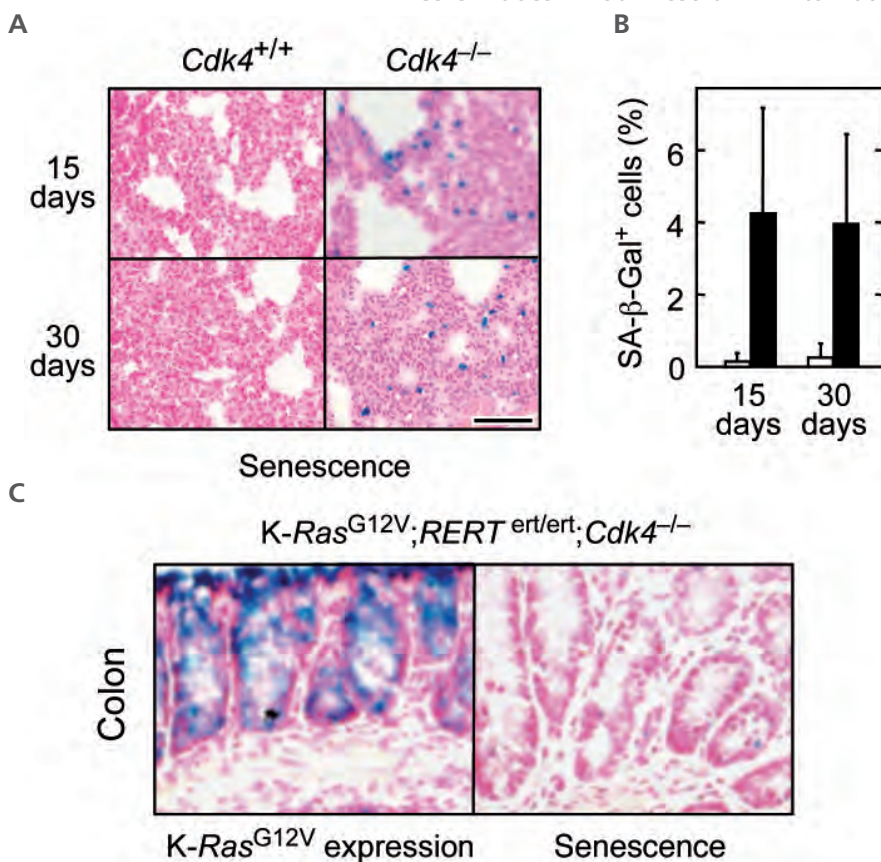


Figure 2: Cdk4 selectively inhibits K-Ras^{G12V}-induced senescence in lung cells. (A) Detection and (B) quantification of senescence-associated β-galactosidase (SA-β-Gal) positive cells in lung sections of *K-Ras^{lox/LSLG12V^{geo}};RERT^{ert/ert};Cdk4^{+/+}* (open bars) and *K-Ras^{lox/LSLG12V^{geo}};RERT^{ert/ert};Cdk4^{-/-}* (solid bars) littermates 15 and 30 days after expression of the resident K-Ras^{G12V} oncogene. (C) Detection of K-Ras^{G12V} expression (based on the surrogate marker β-Geo) (left) and absence of SA-β-Gal⁺ cells (right) in colonic crypts of *K-Ras^{lox/LSLG12V^{geo}};RERT^{ert/ert};Cdk4^{-/-}* mice.

and selective pharmacological inhibition of Cdk4 may provide therapeutic benefit for NSCLC patients carrying K-RAS oncogenes.

CDK inhibitors have fallen out of favour as anti-cancer agents due to their limited activity and high toxicity. However, our results provide genetic and pharmacological evidence indicating that one of the Cdk4 – is essential for proliferation of lung cells, only when

they express a resident K-Ras oncogene. To date, a selective CDK4 inhibitor has shown no significant therapeutic benefit in clinical trials against leukaemias and breast tumours. Our results suggest that this compound as well as novel and more potent CDK4 inhibitors should be tested in clinical trials against K-RAS-driven lung adenocarcinomas.

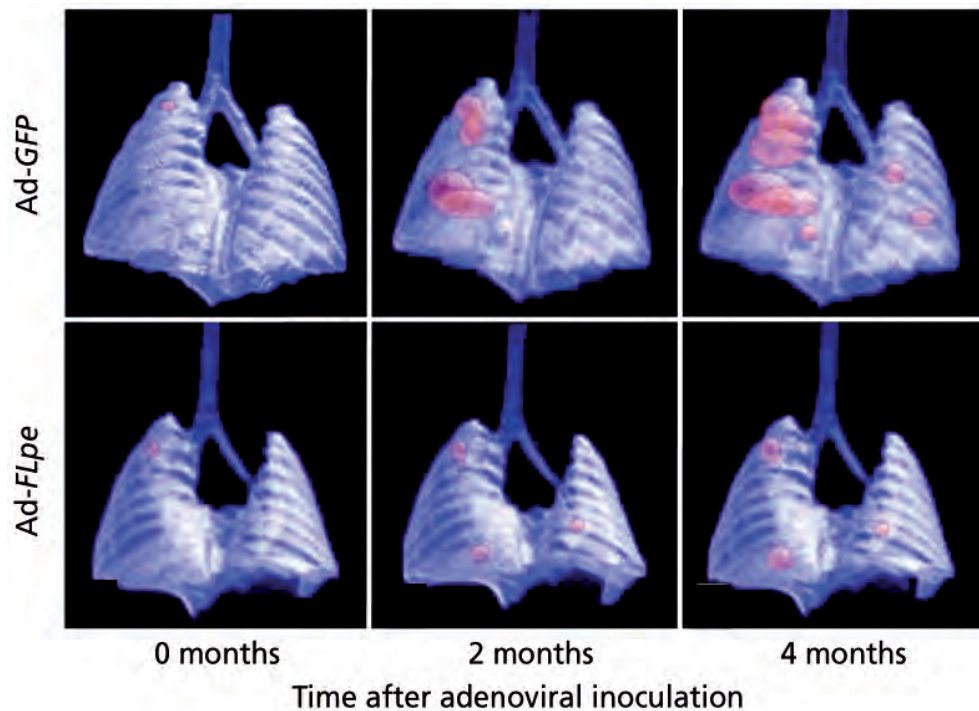


Figure 3: Conditional ablation of Cdk4 expression inhibits progression of K-Ras^{G12V}-driven NSCLC. Three-dimensional reconstruction of CT scans from representative K-Ras^{low/LSLG12V^{geo},RERT^{ert/ert},Cdk4^{fl/fl}} mice treated with adenoviral particles expressing control GFP (Ad-GFP) or Flpe (Ad-FLpe) at the indicated times.

Publications

Malumbres M, Barbacid M (2009). Cell cycle, CDKs and cancer: a changing paradigm. *Nat Rev Cancer* 9, 153-166.

Cerqueira A, Santamaría D, Martínez-Pastor B, Cuadrado M, Fernández-Capetillo O, Barbacid M. (2009). Overall Cdk activity modulates the DNA damage response in mammalian cells. *J Cell Biol* 187, 773-780.

Martínez-Romero C, Rooman I, Skoudy A, Guerra C, Molero X, González A, Iglesias M, Lobato T, Bosch A, Barbacid M, Real FX, Hernández-Muñoz I (2009). The epigenetic regulators Bmi1 and Ring1B are differentially induced in pancreatitis and pancreatic ductal adenocarcinoma. *J Pathol* 219, 205-213.

Urošević J, Sum EY, Moneo V, Drosten M, Dhawahir A, Becerra M, Carnero A, Barbacid M (2009). Using cells devoid of RAS proteins as tools for drug discovery. *Mol Carcinogen* 48, 1038-1047.

Rivera J, Megías D, Navas C, Bravo J (2009). Identification of essential sequences for cellular localization in BRMS1 metastasis suppressor. *PLoS ONE* 4, e6433.

Awards and Recognition

“Distinguished Career Award”, Eli Lilly Foundation, Spain

Member, Board Advisory Group, MRC Cancer Cell Unit, Cambridge, UK

Deputy Editor, *Cancer Research*

Editorial Board Member, *Science Translational Medicine*