

Experimental Oncology Group

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Scientific Report 2010 *crüto*



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 to 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 Cdk (2003-2007).

He has also worked at the NCI campus in Frederick, Maryland (1984-1988) and at the Bristol Myers-Squibb Pharmaceutical Research Institute in Princeton, New Jersey (1988-1998) where he became Vice President of Oncology Drug Discovery. In 1998 he returned to Madrid to create and direct the CNIO (*Cell*, 129: 641-644, 2007).

Mariano Barbacid has authored 251 publications, including 181 original articles and 24 invited reviews in refereed journals as well as 46 book chapters. The average impact factor of his publications in refereed journals is >12. His current Hirsch "h" factor is 88.

He has received several international awards including the Young Investigator Award of the AACR (USA, 1986), the Steiner Prize (Switzerland, 1988), the Ipsen Prize (France, 1994), the *Jiménez Díaz* Award (Spain, 2002), the Brupbacher Cancer Research Prize (Switzerland, 2005), and the Medal of Honour of the International Agency for Research on Cancer (WHO) (France, 2007). He is a member of EMBO (1996) and of the *Academia Europaea* (2004).

Summary

We have made significant inroads in demonstrating that Cdk4 is essential for the development of K-Ras driven non-small cell lung carcinoma (NSCLC). Moreover, we have revealed that K-Ras oncogenic signalling *in vivo* may not conform to the classical Raf/Mek/Erk pathway defined *in vitro*. Systematic ablation of K-Ras downstream kinases has revealed that c-Raf is essential for the onset of NSCLC and cannot be compensated by either B-Raf or A-Raf. Other scientific highlights include the demonstration that pancreatitis-induced inflammation contributes to the progression of pancreatic ductal adenocarcinomas (PDAC) by inhibiting oncogene-induced senescence. The generation of a mouse model for B-Raf induced craneo-facial-cutaneous (CFC) syndrome may help to better understand this disease and to test potential therapeutic strategies.

Strategic Goals

- Understand the role of the Cdk activating kinases (CAK) in the control of the cell cycle
- Identification and characterisation of 'cancer initiating cells' in K-Ras driven NSCLC
- Generation of mouse models for various types of RASopathies
- Elucidation of the role of pancreatitis-induced inflammation in the progression of PDAC
- Validation of molecular targets for therapeutic purposes



Staff scientists: Matthias Drosten, Carmen Guerra and David Santamaría. **Post-doctoral fellows:** Chiara Ambrogio, Sarah Francoz and Raquel García. **Graduate students:** Rafael Blasco, Miguel Ganuza (until August), Isabel Hernández, Sara Mainardi, Carolina Navas, Patricia Nieto and Catherine E. Symonds (since September). **Technicians:** M. Carmen González, Eva Martínez (June-August), Marta San Román and Raquel Villar.

Highlights

Pancreatitis-induced inflammation contributes to pancreatic cancer by inhibiting oncogene-induced senescence

Pancreatic acinar cells of adult mice ($\geq P60$) are resistant to transformation by some of the most robust oncogenic insults including expression of K-Ras oncogenes and loss of *p16Ink4a/p19Arf* or *Trp53* tumour suppressors. These acinar cells however yield pancreatic intraepithelial neoplasias (mPanIN) and ductal adenocarcinomas (mPDAC) when exposed to limited bouts of non-acute pancreatitis – providing they harbour K-Ras oncogenes. Tumour incidence as well as tumour progression increase in the absence of *p16Ink4a/p19Arf* or *Trp53*. Interestingly, adult acinar cells lacking either *p16Ink4a/p19Arf* or *Trp53* do not develop PanINs or any other pre-neoplastic lesions upon induction of pancreatitis thus indicating that K-Ras oncogenes are essential for the initiation of PDAC. In collaboration with Manuel Collado and Manuel Serrano, from the CNIO Tumour Suppression Group, we have also shown that pancreatitis contributes to tumour progression by abrogating the senescence barrier characteristic of low-grade mPanINs (Collado M. et al., *Nature*, 2005). Indeed, none of the low grade PanINs present in K-Ras oncogene bearing mice suffering from pancreatitis displayed senescence markers, including SA- β -Gal and p16Ink4a. However, low grade PanINs present in mice analysed just one month after the cessation of pancreatitis, display senescence markers. A direct connection between pancreatitis and loss of senescence has therefore been established.

We have also evaluated the overall contribution of pancreatitis-induced inflammation to tumor progression. We treated mice with Sulindac, a non-steroidal anti-inflammatory drug thought to act on COX-1 and COX-2 enzymes, for three months straight after cerulein treatment. Mice allowed to recover from pancreatitis displayed the typical lesions induced such as parenchyma atrophy, edema and infiltration of inflammatory cells. Moreover, their pancreata exhibited multiple diffuse PanIN lesions, ranging from low-grade mPanIN1A to invasive PDAC. In contrast, mice exposed to Sulindac had well-preserved pancreata with few areas of parenchyma atrophy and limited infiltration of inflammatory cells. More importantly, we observed a dramatic reduction of up to 75% in the number of high-grade PanIN lesions and PDAC. In addition, the extent of the lesions present in the Sulindac treated animals displayed a dramatic 97% reduction. These results strongly implicate inflammation as a key contributor to the effect of pancreatitis not only in promoting mPanIN formation but also in inducing progression to mPDAC.

In collaboration with Manuel Rodríguez-Justo (University College London) we have demonstrated that this correlation between senescence and pancreatitis-induced inflammation also occurs in human patients. Patients with chronic pancreatitis display senescent PanINs, but only if they have received anti-inflammatory drugs (Figure 1). These results put forward the concept that anti-inflammatory treatment of patients diagnosed with pancreatitis may reduce their risk of developing PDAC.

Generation of mouse models for human RASopathies: a model for cardio-facio-cutaneous syndrome

RASopathies are a class of developmental syndromes that result from congenital mutations in key elements of the RAS/RAF/MEK signalling pathway. A well-recognised RASopathy is the Cardio-Facio-Cutaneous (CFC) syndrome characterised by a distinctive facial appearance, heart defects and mental retardation. Clinically diagnosed CFC patients carry germline mutations in four different genes, *B-RAF*, *MEK1*, *MEK2* and *K-RAS*. *B-RAF* is by far the most commonly mutated locus, displaying mutations that most often result in constitutive activation of the *B-RAF* kinase. This year we have generated and characterised a mouse model for CFC generated by germline expression of a *B-Raf^{ΔSLV600E}* allele that allows very low levels of expression of *B-Raf^{V600E}*, a constitutively active *B-Raf* kinase first identified in human melanoma. *B-Raf^{+/ΔSLV600E}* mice are viable and display several of the characteristic features observed in CFC patients including reduced life span, small size, facial dysmorphism and epileptic seizures. These mice also show upregulation of specific catecholamines and cataracts, two features detected in a low percentage of CFC patients. In addition, *B-Raf^{+/ΔSLV600E}* mice develop neuroendocrine tumours, a pathology not observed in CFC patients. These mice may facilitate a better understanding of the pathophysiology of at least some of the clinical features present in CFC patients. They may also serve as a tool to evaluate the potential therapeutic efficacy of *B-RAF* inhibitors and to establish the precise window at which they could be effective against this congenital syndrome.

Validation of molecular targets for NSCLC: Mek and Erk kinases

We have used genetics to interrogate the role of individual members of the Raf/Mek/Erk cascade in the onset of *K-Ras* oncogene-driven NSCLC. Ablation of *Erk1* or *Erk2* in *K-Ras* oncogene expressing lung cells had no significant effect. Yet, elimination of both *Erk* kinases completely blocked tumour development. This suggests that *Erk* kinases have full compensatory activities to allow at least the initiation of NSCLC. Similar results were obtained with *Mek* kinases. Ablation of either *Mek1* or *Mek2* had no effect on tumour development however ablation of *Mek1* in mice lacking *Mek2* completely prevented NSCLC. Unfortunately, systemic ablation of both *Erk* kinases in young mice induced multiple organ failure resulting in the death of all mice within 3 weeks of tamoxifen treatment to remove the conditional *Erk2* floxed alleles (*Erk1* was deleted in the germline). DNA analysis of tissues obtained from moribund animals revealed recombination rates of the *Erk2^{lox}* alleles ranging from 40% to 60%. This therefore indicates that complete loss of *Erk* kinases even in a limited number of cells is incompatible with adult life. Likewise, when *Mek1^{lox/lox};Mek2* expressing an ubiquitous CreERT2 recombinase were exposed to a tamoxifen diet at 30 days of age, they displayed a rapid deterioration in health leading to death just 2 weeks after starting the tamoxifen diet.

Southern blot analysis of DNA isolated from tissues from sick/moribund animals revealed recombination rates of *Mek1^{lox}* alleles ranging from 70% to 80% in most tissues. Necropsy analysis of moribund mice

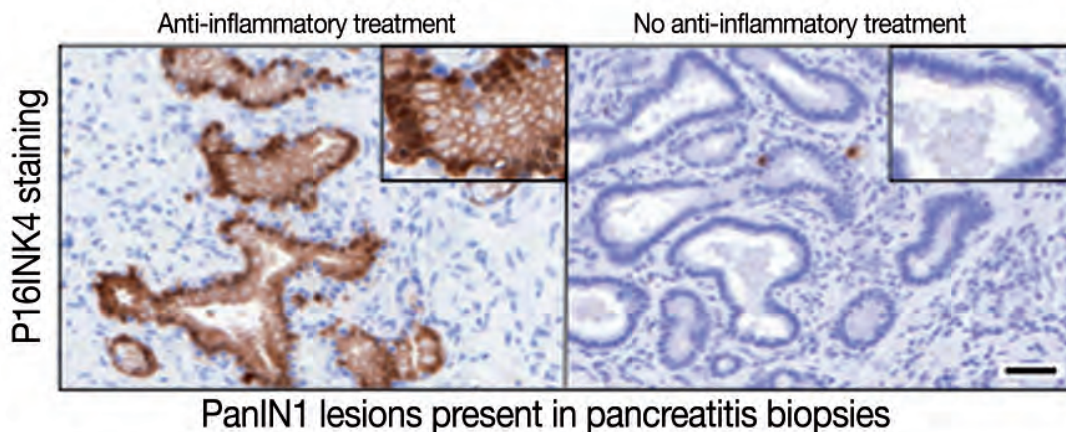
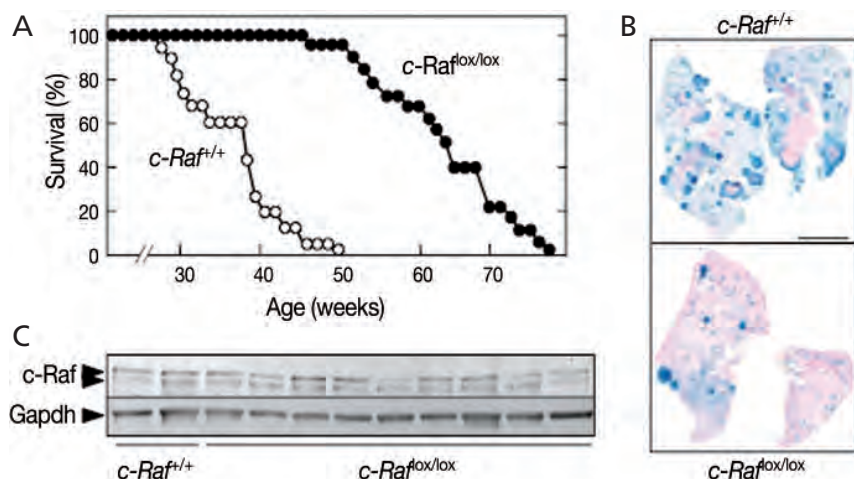


Figure 1: Senescence markers in human low-grade PanINs. P16INK4a immunostaining of biopsies obtained from patients suffering from chronic pancreatitis showing PanIN lesions from a patient (left) treated or (right) non-treated with anti-inflammatory drugs. Insets show amplified images to illustrate nuclear staining in PanIN1 lesions. Scale bar represents 50 μ m.

Figure 2: c-Raf is essential for K-Ras^{G12V} induced NSCLCs in mice. (A) Survival of K-Ras^{G12V};c-Raf^{+/+} (n=22) (open circles) and K-Ras^{G12V};c-Raf^{lox/lox} (n=23) (solid circles) mice treated with Ad-Cre at 8 weeks of age. (B) Whole mount X-Gal staining of lung sections from mice 6 months after Ad-Cre treatment. Scale bar, 5 mm. (C) Western blot analysis of c-Raf expression in lysates obtained from individual tumours collected after Ad-Cre treatment. Please note that all tumours express c-Raf. Gapdh was used as loading control.



revealed multiple defects, including severe alterations in the structure of intestinal and colonic tissue incompatible with life. Even limited ablation of Mek alleles resulted in significant alterations of the normal architecture including distorted crypts, blunted and shorter villi, increased lamina propria and goblet cell hyperplasia. Likewise, the colonic tissue displayed loss as well as severe shortening of the crypts.

Validation of molecular targets for NSCLC: Raf kinases

Unlike the results obtained with the Mek and Erk kinases, no such compensatory activities were observed among Raf kinases. While ablation of B-Raf had no effect on K-Ras

oncogene-driven tumour development, c-Raf expression was essential for the onset of NSCLC (Figure 2). Interestingly, selective ablation of B-Raf or c-Raf kinases in young mice had no deleterious consequences for normal homeostasis. More importantly, when we exposed B-Raf^{lox/lox};c-Raf^{lox/lox} mice to a tamoxifen diet for three months, they remained healthy and did not show weight loss or behavioural changes. Histological examination of twenty different tissues at the end of the treatment did not reveal detectable abnormalities. These tissues displayed efficient recombination of the B-Raf^{lox} and/or c-Raf^{lox} alleles, ranging from 80% to 100% excision in the case of B-Raf^{lox} and 60% to 100% in the case of c-Raf^{lox}.

These results indicate that concomitant elimination of c-Raf and B-Raf in adult mice is well tolerated. Analysis of these tissues for the expression of A-Raf indicated that this isoform is widely expressed regardless of the ablation of B-Raf and c-Raf alleles. Thus, suggesting that A-Raf may compensate for the absence of the other Raf isoforms. Whether ablation of the three Raf loci will be compatible with adult homeostasis remains to be determined. These observations do however indicate that c-Raf plays a unique role in mediating K-Ras signalling and makes it a possible target for therapeutic intervention.

Publications

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