EXPERIMENTAL ONCOLOGY GROUP

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OVERVIEW

KRAS oncogenes have been identified in at least one fifth of all human cancers. In spite of recent successes with checkpoint inhibitors, most *KRAS* mutant tumours, including lung adenocarcinomas, are still treated with cytotoxic compounds approved over 2 decades ago. Moreover, attempts to block KRAS oncogenic activity with selective inhibitors of the MEK kinase, a downstream effector, have turned out to be major failures. Two MEK inhibitors, Trametinib and Selumetinib, have failed to show significant anti-tumour activity in large phase III clinical trials due to unacceptable toxicities. In our laboratory, we have continued our quest to validate therapeutic targets using a new generation of genetically engineered mouse tumour models that allow us to evaluate their anti-tumour properties as well as their potential toxic effects in tumour-bearing mice. These studies have allowed the identification of c-RAF as a target capable of inducing significant tumour regressions in advanced KRAS/TRP53 mutant lung tumours without inducing major toxicities. These observations suggest that forthcoming c-RAF inhibitors may provide significant therapeutic benefits in the clinic.

"Systemic ablation of c-RAF induces regression of a significant percentage of advanced K-*Ras*/*Trp*53 mutant lung adenocarcinomas by a mechanism independent of MAPK signalling that results in the induction of acceptable toxicities."

RESEARCH HIGHLIGHTS

Regression of advanced K-Ras/Trp53 mutant lung adenocarcinomas upon systemic ablation of c-RAF expression

Almost a quarter of all solid tumours harbour K-*RAS* oncogenes. Yet, more than 30 years after their identification in human cancer, there are no selective therapies to treat these tumours. Inhibitors of K-RAS oncogenes activated by G12C mutations, a mutation frequently identified in lung adenocarcinomas, have already entered clinical trials. Yet, direct targeting of other mutations has proven to be challenging. Genetic interrogation of the MAPK pathway revealed that systemic ablation of Mek or Erk kinases in adult mice prevent tumour development but are unacceptably toxic. This year, we demonstrated that ablation of c-Raf expression in advanced mouse lung tumours driven by K-*Ras*^{G12V}/*Trp53* mutations led to significant tumour regression with no detectable appearance of resistance mechanisms (FIGURE 1). Tumour regression results from massive apoptosis. Importantly, systemic abrogation of c-Raf expression does not inhibit canonical MAPK signalling, hence, resulting in limited toxicities. These observations suggest that therapeutic strategies aimed at inhibiting c-RAF kinase activity may not be suitable since they may only block MAP Kinase activation. Indeed, three independent c-RAF kinase inhibitors have shown to be rather toxic even at non-therapeutic doses. Therefore, we need to explore other therapeutic strategies. Drugs capable of degrading the c-RAF protein could be most effective in the clinic. Yet, drugs that promote protein degradation are still at very early stages of development. Inhibition of c-RAF by small interfering RNA is another possible approach, but still faces significant technical challenges. Finally, selective targeting c-RAF effectors regulated through non-kinase mechanisms such as ROKalpha, ASK1 and MST2 will also be challenging since they will require to be activated. As Frank MacCormick put it in a Preview that accompanied our *Cancer Cell* paper (San Clemente *et al*., 2018) "In the effort to harness c-Raf biology to target KRAS mutant cancers, the goal posts seemed to have moved again". In any case, our results should provide the experimental bases to design novel c-RAF based therapeutics to treat K-RAS mutant cancers.

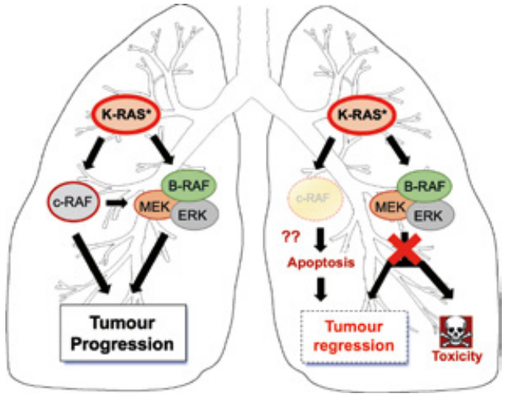

Figure 1 Systemic ablation of c-RAF expression results in equal or better levels of tumour regression while inducing acceptable toxicities. In contrast, inhibition of the MAP Kinase pathway induces tumour regression but with unacceptable toxicities both in mice (Blasco *et al.*, *Cancer Cell* 2011) and in patients (Trametinib and Selumetinib failed clinical trials).

SAA3 is a key mediator of the pro-tumorigenic properties of cancer associated fibroblasts in pancreatic ductal adenocarcinomas

Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant human tumours for which there are no efficacious therapeutic strategies. This tumour type is characterised by an abundant desmoplastic stroma that promotes tumour progression. It is generally accepted that cancer-associated fibroblasts (CAFs) stimulate tumour progression and might be implicated in drug resistance and immunosuppression. Yet, recent studies have shown that physical or genetic elimination of the stroma leads to more aggressive tumour development. In an attempt to clarify the role of the desmoplastic stroma in PDAC development and progression, we decided to reprogram the CAFs that make up the stromal tissue by identifying, and subsequently targeting, genes responsible for their pro-tumorigenic properties. First, we compared the transcriptional profile of PDGFRα+ CAFs isolated from genetically engineered mouse PDAC tumours with that of normal pancreatic fibroblasts (NPFs) in order to identify genes potentially implicated in their pro-tumorigenic properties. We have observed that the most differentially expressed gene, *Saa3*, a member of the acute-phase Serum Amyloid A (SAA) apolipoprotein family, is a key mediator of the pro-tumorigenic activity of PDGFRα+ CAFs. Whereas *Saa3* competent CAFs stimulate the growth of PDAC tumour cells in an orthotropic model, *Saa3 null* CAFs inhibit tumour growth. Saa3 also plays a role in the cross-talk between CAFs and tumour cells (FIGURE 2). Ablation of *Saa3* in pancreatic tumour cells makes them insensitive to the inhibitory effect of *Saa3 null* CAFs. As a consequence, germline ablation of *Saa3* does not prevent PDAC tumour development in mice (FIGURE 2). The pro-tumorigenic activity of Saa3 in CAFs is mediated by Mpp6, a member of the palmitoylated membrane protein subfamily of the peripheral membrane-associated guanylate kinases. Finally, we interrogated whether these observations could be translated to a human scenario. Indeed, *SAA1*, the orthologue of murine Saa3, is overexpressed in human CAFs. Moreover, high levels of *SAA1* in the stromal component correlate with worse survival. These findings support the concept that selective inhibition of SAA1 in CAFs may provide potential therapeutic benefit to PDAC patients. ■

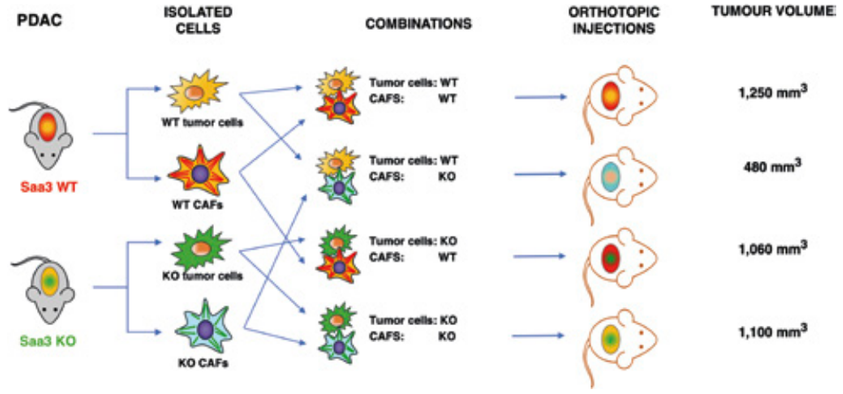

Figure 2 Crosstalk between pancreatic tumour cells and CAFs in the presence and absence of Saa3. Diagram depicting the *in vivo* orthotopic tumour assays in immunodeficient mice carried out to determine the pro-tumorigenic properties of *Saa3* competent (WT) (red) and *Saa3 null* (KO) (light blue) CAFs on pancreatic tumour cells isolated from *Saa3* competent (WT) (yellow) and *Saa3 null* (KO) (green) tumours.

PUBLICATIONS

- Sanclemente M, Francoz S, Esteban-Burgos L, Bousquet-Mur E, Djurec M, Lopez-Casas PP, Hidalgo M, Guerra C, Drosten M, Musteanu M, Barbacid M (2018). c-Raf ablation induces regression of advanced K-Ras/Trp53 mutant lung adenocarcinomas by a mechanism independent of MAPK signaling. *Cancer Cell* 33, 217-228.
  Commentaries for this article appeared in:
  - Cancer Cell: F. McCormick. c-Raf in KRas mutant cancers: a moving target. *Cancer Cell* 33, 158-159.
  - Science Translational Medicine: A. Lujambio. A new hope for KRAS mutant cancers. *Sci Transl Med* 10, eaas8964. http://stm.sciencemag.org/content/10/429/eaas8964.full.

  This article was also recommended in F1000Prime as being of special significance in its field.
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PATENT

- Barbacid M, Guerra C, Blasco MT, Navas C (2018). Combined therapy against cancer. *EP18382555.3*.

AWARDS AND RECOGNITION

Mariano Barbacid:
- *Premio Fulbright*, Madrid, Spain.
- JC Bose Memorial Lecture, Saha Institute of Nuclear Physics, Kolkata, India.
- *Severo Ochoa* Plenary Lecture, XLI Meeting of the Chilean Society for Biochemistry and Molecular Biology, Iquique, Chile.
- Maimonides Memorial Lecture, Córdoba, Spain.
- Keynote Speaker, AACR Special Conference: Targeting RAS-Driven Cancers, San Diego California, USA.
- Chair, Plenary Symposium on "Genetic Background Matters", 25th Biennial Congress of the European Association for Cancer Research, Amsterdam, The Netherlands.
- Chair, EMBO Workshop on "Cellular Signalling and Cancer Therapy", Cavtat, Croatia.

Carmen Guerra:
- *III Beca Carmen Delgado/Miguel Pérez-Mateo*.
- CNIO Award for Excellence in Research by Staff investigators.

- Manuel Sanclemente: Award for PhD-Authored Publications, CNIO Lab Day.