KRAS oncogenes have been identified in one fifth of all human cancers. Yet, no selective inhibitors have been approved so far. Moreover, attempts to block KRAS oncogenic activity with selective inhibitors of KRAS signalling pathways have failed so far due to unacceptable toxicities. In our laboratory, we have continued our quest to validate therapeutic targets for KRAS driven lung and pancreatic tumours 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 RAF1 as the only target within the MAPK signalling pathway capable of inducing significant tumour regressions in advanced KRAS/TRP53 mutant tumours without inducing major toxicities. We are now focusing our research interests on: (i) devising therapeutic strategies to selectively targeting RAF1 and (ii) identifying additional targets that may cooperate with RAF1 inhibition, with the ultimate goal of translating these results to the clinic.

“We have achieved, for the first time, the complete regression of a significant percentage of advanced K-Ras/Trp53 mutant pancreatic ductal adenocarcinomas by inducing the systemic ablation of combined RAF1 and EGFR expression.”
Complete regression of advanced pancreatic ductal adenocarcinomas upon combined inhibition of EGFR and RAF1 expression

We have developed a new GEM strain that separates temporally and spatially tumour development from target ablation/inhibition. This strain, K-RasG12V/prom1; Tp53fl/fl; O-FlpO;O-FlpO;Tg;UBC-CreERT2; designated KPeFC, incorporates 2 distinct recombinases, FlpO and CreERT2. FlpO is responsible for tumour induction, whereas expression of the tumoxin (TMX) inducible CreERT2 recombinase is driven by the human Ubiquitin C promoter (hUBC). Thus, exposure of KPeFC mice to a TMX-containing diet allows the systematic recombination of any conditional flxed allele added to this strain. We used this strain to interrogate the therapeutic properties of ablating RAF1 and EGFR based on previous observations using initiation models. Tumour-bearing KPeFC;EGFRlox/lox;c-RAFlox/lox mice were exposed to a TMX-containing diet. Eight of 12 KPeFC;EGFRlox/lox;c-RAFlox/lox mice displayed a rapid decrease in tumour volume upon TMX exposure. Six of these mice became tumour-free by micro-ultrasound analysis after 6 weeks of TMX exposure. Four mice were sacrificed after 6 weeks of TMX exposure, whereas the remaining animals were allowed to survive for 10 additional weeks. No tumour reappearance was observed during this time period. Moreover, detailed histological examination of their pancreata revealed normal tissue architecture, indicating that these tumours had completely regressed. Many therapies fail in the clinic due to unacceptable toxic effects. However, systemic deletion of EGFR and RAF1 expression led to acceptable toxicities, all induced by loss of EGFR expression, mainly involving skin alterations such as hyperplasia and disorganisation of the epidermis, hyperkeratosis, folliculitis and inflammation with increased numbers of mast cells and significant hair loss. These skin defects are highly reminiscent of the acneiform rash and folliculitis observed in human PDAC tumours. These observations suggest that combined inhibition of EGFR and c-RAF expression may have significant therapeutic activity in human PDAC tumours.

Gene Set Enrichment Analysis (GSEA) identified several pathways enriched in the resistant tumour cells, including those corresponding to ‘E2F targets’, ‘EMT’ and ‘MYC targets’. Other enriched pathways included the ‘PI3K/AKT/mTOR’ and ‘E2/RAS/STAT3’ signalling pathways. Comparison of data obtained by RNAsig analysis with a transcriptional classification of human PDACs (Bailey et al., 2016) revealed that NC cells displayed a transcriptional profile most similar to the ‘squamous subtype’.

Finally, we determined whether combined inhibition of EGFR and c-RAF signalling could provide therapeutic benefit to PDAC patients. To this end, we knocked down their expression in cells derived from 10 PDIX tumour models harbouring K-RAS and TRP53 mutations. Individual knockdown of EGFR or c-RAF expression reduced their proliferative properties to various extents. More importantly, combined knockdown of EGFR and c-RAF expression completely interfered with the proliferative capacity of those cells derived from 9 out of the 10 PDIX tumour models. Four of the PDIX-derived tumour cells that fully responded to EGFR or c-RAF knockdown were injected into immunocompromised mice. Again, only the combined knockdown of EGFR and c-RAF effectively inhibited growth of these human PDAC tumour cells in vivo. These observations suggest that combined inhibition of EGFR and c-RAF expression may have significant therapeutic activity in human PDAC tumours.

PUBLICATIONS


PATENT


AWARDS AND RECOGNITION

Honorary Member, American Academy of Pharmacy, Granada.

Honorary Member, Spanish Society of Biochemistry and Molecular Biology (SEBBM), Madrid.

Amores Gold Award, Córdoba.

ECC Award to a Cancer in Oncology Research, ECO Foundation, Madrid.

Health Award (Premio a la Salud), Fundación Gala, Madrid.

Best researcher of 2019 (Investigador del año), La Rioja, Madrid.

Keynote speaker, BioCity Turku Frontiers of Science, Turku, Finland.

Char-RAD-Targeted Drug Discovery Summit, Boston, USA.

Opening Lecture, NCR Cancer Conference, Glasgow, UK.

Keynote Lecture, CBERCROP workshop on Preclinical Models for Translational Research, Barcelona.

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