GENOMIC INSTABILITY GROUP

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OVERVIEW

The Genomic Instability Group focuses its research on understanding the molecular mechanisms leading to cancer and other age-related diseases, with the ultimate goal of translating this knowledge into novel therapeutic strategies. Our initial investigations centred on Replicative Stress (RS), a type of DNA damage sensed by the ATR kinase, and that is particularly abundant in some cancer cells. Our work in this area led to the discovery of selective ATR inhibitors that were further improved to enable their clinical development as anticancer agents. Next, we became increasingly interested in understanding the mechanisms of drug resistance to specific agents, such as inhibitors of ATR or USP7. More recently, our group has revealed that one of the most frequent mutations in human cancer, inactivation of the tumour suppressor FBXW7, leads to multidrug resistance (MDR). Importantly, we have also discovered strategies to overcome MDR, which is an important area of our current research.

"We have discovered a new mechanism that drives multidrug resistance. In addition, we have shown that the depletion of PD-L1 expressing cells might be a fruitful approach for cancer therapy."

RESEARCH HIGHLIGHTS

The Integrated Stress Response as a vulnerability of cancer cells

Last year, we reported that *FBXW7* mutations lead to multidrug resistance (MDR), limiting the efficacy of most available antitumor agents. Importantly, FBXW7 is one of the 10 most frequently mutated genes in cancer due to either inactivating mutations and/or allelic loss. Furthermore, mutations in this gene are among the most significantly associated with poor survival across all human cancers. Interestingly, we discovered that, despite their MDR phenotype, FBXW7 deficient cells were preferentially sensitive to therapies targeting mitochondria, such as the antibiotic tigecycline. Subsequently, we identified that that the toxicity of tigecycline for cancer cells is mediated by the Integrated Stress Response (ISR). In support of this, nuclear accumulation of ATF4, one of the hallmarks of ISR activity, was induced by tigecycline and reverted by the ISR inhibitor ISRIB. Moreover, and by searching for additional compounds that could target FBXW7 deficient cells, we found another set of seemingly unrelated compounds that did so, all of which activated the ISR (FIGURE 1). Surprisingly, these compounds were already known to have antitumor effects through very different mechanisms of action, such as inhibition of B-RAF or EGFR. This raises the important question as to what extent the anticancer effects of several clinically used drugs might be partly mediated by a previously unknown effect of these compounds in activating the ISR. We are currently investigating the basis of these observations, as they suggest the exciting possibility that a targeted activation of the ISR might be able to trigger cell death in cancer cells that are otherwise resistant to other chemotherapies.

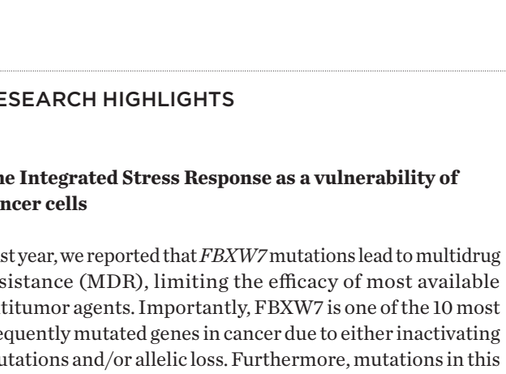

FIGURE 1 Multidrug resistance of FBXW7-deficient cells can be overcome by activation of the ISR. (**A**) Sensitivity of FBXW7-deficient DLD-1 to the ISR-activating drug tunicamycin. (**B**) Drugs that are similar to the antibiotic tigecycline, based on comparison of transcriptional signatures available at the connectivity map (CMAP). Note the presence of several seemingly unrelated tyrosine kinases in this set, all of which trigger a similar transcriptional signature to well established ISR-inducers such as tunicamycin or salubrinal. (**C**) Nuclear levels of ATF4 as evaluated by High-Content Microscopy in DLD-1 cells. Note that compounds to which FBXW7-deficient cells are resistant do not activate the ISR.

Targeting PD-L1 expressing cells in cancer therapy

The latest advances in immunotherapy for the treatment of cancer have incredibly improved the prognosis of a wide range of malignancies. Not surprisingly, the discovery of the immune checkpoint mediated by PD-1 and CTLA-4 receptors and of how targeting these pathways can be exploited for cancer therapy was awarded the Nobel Prize in Medicine in 2018. Antibodies targeting the PD-1/PD-L1 interaction are among the most widely used immunotherapy strategies, but, despite the indisputable success of these treatments, only 20-40% of the patients respond, and even fewer show durable responses. We hypothesised that the elimination of PD-L1 expressing cells, which may display additional checkpoint mediators on their membranes, could have broader antitumoral effects than targeting only the PD-1/PD-L1 interaction. To address this, we generated mice carrying an inducible suicidal reporter allele of PD-L1, which allows the isolation and identification of PD-L1-expressing cells, as well as their selective elimination upon treatment with an otherwise inert compound. Our work with these mice has revealed that the depletion of PD-L1 positive cells potentiates immune responses against different stimuli, such as a septic cytokine storm. In the context of cancer, we found that depletion of PD-L1-expressing cells favoured the clearance of tumour cells in a mouse model of peritoneal metastasis and, consequently, prolonged the survival of the animals (FIGURE 2). This work supports the usefulness of targeting PD-L1^{+} cells in cancer therapy, and provides the immunotherapy research community with a useful genetic tool for further investigations of the PD-1/PD-L1 checkpoint. ■

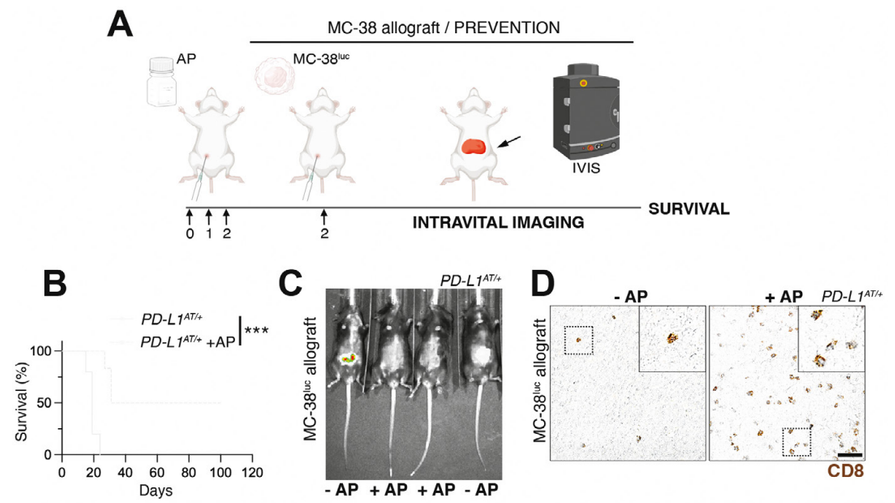

FIGURE 2 Depleting PD-L1^{+} cells prolongs survival in an immunocompetent model of peritoneal cancer metastasis. (**A**) MC-38 cells were intraperitoneally injected into mice previously treated with AP20187 to deplete PD-L1^{+} cells. (**B**) Survival curve of control and AP20187-treated *PD-L1*$^{AT/+}$ mice after i.p. inoculation of MC-38 allografts. (**C**) Representative IVIS from the experiment defined in (**B**) at day 4 post-tumour injection. (**D**) IHC of CD8 in intraperitoneal MC-38 allografts isolated from control and AP20187-treated *PD-L1*$^{AT/+}$ mice. Insets illustrate the presence of tumour-infiltrating CD8^{+} cells.

PUBLICATIONS

- Sanchez-Burgos L, Navarro-González B, García-Martín S, Sirozh O, Mota-Pino J, Fueyo-Marcos E, Tejero H, Antón ME, Murga M, Al-Shahrour F, Fernandez-Capetillo O (2022). Activation of the integrated stress response is a vulnerability for multidrug-resistant FBXW7-deficient cells. *EMBO Mol Med* 14, e15855.
- Corman A, Sirozh O, Lafarga V, Fernandez-Capetillo O (2022). Targeting the nucleolus as a therapeutic strategy in human disease. *Trends Biochem Sci*. PMID: 36229381.
- Hühn D, Martí-Rodrigo P, Mouron S, Hansel C, Tschapalda K, Porebski B, Häggblad M, Lidemalm L, Quintela-Fandino M, Carreras-Puigvert J, Fernandez-Capetillo O (2022). Prolonged estrogen deprivation triggers a broad immunosuppressive phenotype in breast cancer cells. *Mol Oncol* 16, 148-165.
- Murga M, Fernandez-Capetillo O (2022). Emerging concepts in drug discovery for cancer therapy. *Mol Oncol* 16, 3757-3760.
- Egea J, López-Muñoz F, Fernández-Capetillo O, Reiter RJ, Romero A (2022). Alkylating agent-induced toxicity and melatonin-based therapies. *Front Pharmacol* 13, 873197.
- Sanchez-Burgos L, Gómez-López G, Al-Shahrour F, Fernandez-Capetillo O (2022). An in silico analysis identifies drugs potentially modulating the cytokine storm triggered by SARS-CoV-2 infection. *Sci Rep* 12, 1626.
- Colicchia V, Häggblad M, Sirozh O, Porebski B, Balan M, Li X, Lidemalm L, Carreras-Puigvert J, Hühn D, Fernandez-Capetillo O (2022). New regulators of the tetracycline-inducible gene expression system identified by chemical and genetic screens. *FEBS Open Bio* 12, 1896-1908.
- Galindo-Campos MA, Lutfi N, Bonnin S, Martínez C, Velasco-Hernandez T, García-Hernández V, Martín-Caballero J, Ampurdanés C, Gimeno R, Colomo L, Roué G, Guilbaud G, Dantzer F, Navarro P, Murga M, Fernández-Capetillo O, Bigas A, Menéndez P, Sale JE, Yélamos J (2022). Distinct roles for PARP-1 and PARP-2 in c-Myc- driven B-cell lymphoma in mice. *Blood* 139, 228-239.
- Fueyo-Marcos E, Fustero-Torre C, Lopez G, Antón ME, Al-Shahrour F, Fernández-Capetillo O, Murga M (2022). PD-L1 ATTAC mice reveal the potential of targeting PD-L1 expressing cells in cancer therapy. *bioRxiv*. doi: https://doi.org/10.1101/2022.07.22.501095.

Book Chapter

- Sirozh O, Saez-Mas A, Lafarga V, Fernandez-Capetillo O (2022). Basic concepts and emergent diseases mechanisms of amyotrophic lateral sclerosis. In Bradshaw RA, Hart GW, Stah PD (Eds), *Encyclopedia of Cell Biology Second Edition* (pp. 644-665). Academic Press – Elsevier Inc. https://doi.org/10.1016/B978-0-12-821618-7.00266-2.