GENOMIC INSTABILITY GROUP

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Titulado Superior (Advanced Degree)

OVERVIEW

The Genomic Instability Group centres its research on understanding how cells respond to DNA damage, in particular to a specific type of harm known as replication stress (RS). Oncogene-induced RS has been confirmed as the main source of genomic rearrangements in cancer cells. In mammals, RS triggers a cellular response initiated by ATR and CHK1 kinases, known as the Replicative Stress Response (RSR). Throughout the years, our laboratory has developed a wide battery of cellular and animal tools for the study of the RSR. Among them, we have mice with enhanced or limited function of ATR and CHK1 kinases, cell lines in which the RSR can be activated at will and chemical inhibitors of ATR. Our studies have enhanced our understanding of the impact of RS on cancer and ageing, and have provided novel drugs with antitumoural potential that exploit the presence of RS in cancer cells. Overall, our goal is to understand the molecular mechanisms governing genome protection and repair – particularly during replication – and to exploit this knowledge as a way to fight against cancer.

"In 2018 we have, among other achievements, extended our line of research on the mechanisms of resistance to cancer therapies and revealed a novel mechanism by which cells couple DNA replication termination to mitotic entry."

RESEARCH HIGHLIGHTS

Coupling DNA replication termination to mitotic entry

The cell cycle consists of a sequence of ordered events leading to the duplication of DNA and ultimately cell division. According to this model, once DNA replication is finished, there is a G2 transition phase that precedes mitosis. However, how and when mitosis is triggered is not yet fully understood. In fact, it has been long speculated that some yet-to-be-discovered checkpoint prevents mitotic entry until DNA replication is completed. We previously described that inhibition of the USP7 deubiquitinase leads to the ubiquitination of replisome components and replication termination. We have now seen that DNA replication triggered by USP7 inhibitors occurs concomitant to a generalised activation of the mitotic kinase CDK1 throughout the entire cell cycle, which impairs chromosome segregation and is toxic for mammalian cells. Accordingly, the toxicity of USP7 inhibitors is alleviated by CDK1 inhibition. Besides from its interest in clarifying how USP7 inhibitors kill cells, this work provides direct evidence for the existence of a ubiquitin-based signalling code that couples DNA replication termination to mitotic entry.

Rescuing RAS deficiency in mammalian cells

Previous work in our laboratory revealed the mechanisms of resistance to genotoxic anticancer agents such as ATR inhibitors. We have now looked into potential mechanisms of resistance to targeted therapies. In the context of targeted therapies, RAS is assumed to be the 'Holy Grail'. More than 30% of all human cancers are driven by mutations in the RAS family of genes and, if not RAS, another member of the pathway is frequently altered. Since RAS inhibitors have been technically very difficult to develop, most drugs that have reached the clinic actually target some other components of the signalling route such as EGRF, RAF or MEK. However, all of these targeted therapies invariably confront the emergence of resistance. To which extent resistance would also occur to RAS inhibitors remains unknown. In this regard, we have recently identified a mutation – the loss of the ETS-domain factor *ERF* – that enables the growth and differentiation of mouse embryonic stem cells (mESC) lacking all RAS genes (H-, N- and K-*Ras*). Strikingly, ERF deficiency supports the generation of RAS-less teratomas, this being the first example of a tumour that can develop in the absence of RAS proteins. We believe this work indicates that, even if potent and selective inhibitors of RAS are finally developed, they might likely confront resistance through mutations of other genes such as *ERF*. We are currently investigating the role of ERF and other ETS-domain factors in the context of resistance to targeted therapies in cancer. ■

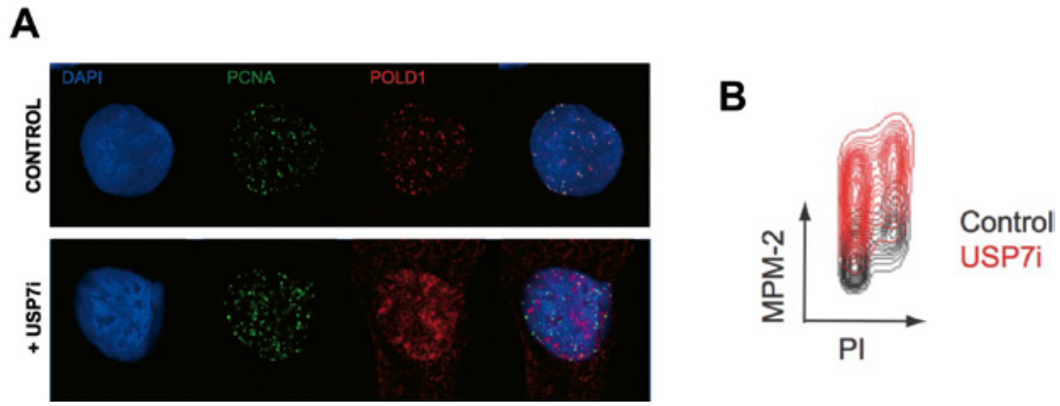

Figure 1 USP7 coordinates DNA replication with mitotic entry. (**A**) Immunofluorescence of PCNA (green) and POLD1 (red) illustrating the effects of USP7 inhibition (USP7i) in replisome disassembly in MEF. DNA was stained with DAPI (blue). (**B**) Flow cytometry measuring CDK activity (MPM-2) and DNA content (PI) in cells exposed to USP7i, illustrating the generalised activation of CDK activity throughout the cell cycle in response to the inhibitor.

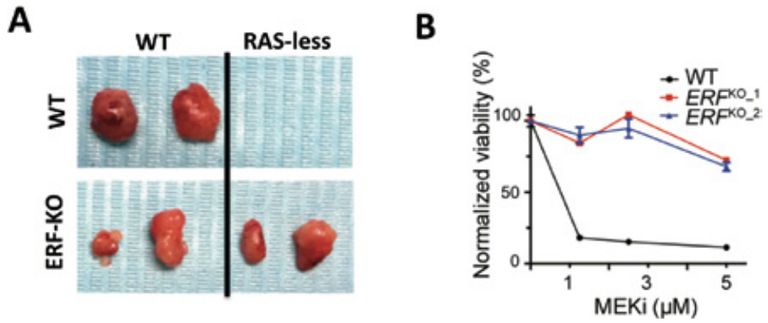

Figure 2 ERF deficiency is synthetically viable with the absence of RAS signalling. (**A**) Representative image of teratomas that are observed after injecting nude mice with mESC of the indicated genotypes (RAS-less = deficient in H-, N- and K-*Ras*). (**B**) Cell-Titer Glo assay in leukaemia-derived KBM7 cells, illustrating the increased resistance of *ERF*-deficient cells to MEK inhibition.

PUBLICATIONS

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