The main goal the Genomic Instability Group is to understand the molecular mechanisms underlying cancer and other age-associated diseases, with the ultimate objective of translating this knowledge into effective treatments for patients. To this end, we have developed over the years several molecular tools and in vivo models, which have led us to make important progress in basic as well as in translational research. Among other achievements, we have extensively studied the molecular mechanisms by which cells duplicate and repair their genomes, developed new inhibitors that can be used for targeted cancer therapy, and created mouse models that revealed the physiological consequences of genomic instability. More recently, we have developed an interest in exploring the mechanisms of drug resistance in cancer therapy and how to overcome this problem in cancer, as well as in other age-related diseases lacking a cure, such as neurodegeneration.

“In 2021 we discovered that one of the most frequent mutations in cancer drives multidrug resistance and advanced in our understanding of how replisomes are dissolved once DNA replication ends.”
RESEARCH HIGHLIGHTS

Overcoming multi-drug resistance associated to FBXW7 deficiency

Drug resistance is a huge medical challenge. In cancer, it is estimated that resistance to therapy accounts for almost 90% of treatment failures. Thus, there is an urgent need to obtain a better understanding of the mechanisms behind drug resistance, which are still rather fragmented. Over the last few years, our laboratory has developed strong expertise in genome-wide CRISPR screens to identify mutations related to resistance to different cancer treatments. Throughout the course of these studies, we found sgRNAs targeting FBXW7 as recurrent hits that provided resistance to many independent drug combinations. In this respect, we later confirmed that FBXW7 deficiency indeed confers resistance to the vast majority of currently used antitumour agents. These observations were supported by in silico analyses indicating that FBXW7 deficiency is among the most significant multidrug resistance mutations that can be detected. Of note, FBXW7 is among the top 10 most mutated genes in cancer, highlighting the relevance of our discovery. Importantly, in our study we also identified that, despite their multidrug resistance, FBXW7 deficient cells were preferentially vulnerable to treatment with therapies that target mitochondrial activity, such as the antibiotic tigecycline (FIGURE 1). In summary, this work has revealed that one of the most frequent mutations in cancer is associated to multidrug resistance and provided some initial ideas about how this resistance might be overcome.

Extracting replisome components from chromatin when DNA replication ends

DNA replication, carried out by a large protein complex known as the replisome, requires finely-tuned regulatory mechanisms that often involve post-translational modifications such as SUMOylation or ubiquitination. A few years ago, while performing one of the first proteomic characterisations of the human replisome, our group found an intriguing feature: whereas replisomes are SUMO- and ubiquitin-low environments, mature non-replicating chromatin displays the opposite trend. Later, we discovered that USP7 inhibition mimics the end of DNA replication and simultaneously drives CDK1 activation in such a manner that is toxic and that can help to understand the antitumour effects of USP7 inhibitors. During 2021, we resolved what happens to the replisome components that become ubiquitinated upon USP7 inhibition. We now know that these factors become extracted from chromatin by the segregease VCP, which uses FAP1 as an adaptor to bind SUMOylated and ubiquitinated factors (FIGURE 2). Noteworthy, equivalent conclusions were drawn from a genetic screen completed in C. elegans by our collaborator Thorsten Hoppe, highlighting the evolutionary conservation of this pathway. In this line of research, we are now particularly intrigued by the mechanisms that drive CDK1 activation upon the completion of DNA termination, as we believe this is still one of the key missing pieces from our basic understanding of the cell cycle.

FIGURE 1

(a) Overcoming multidrug resistance. (A) Effects of the indicated drug on the viability of FBXW7 wild-type and knockout DLD-1 human colon adenocarcinoma-derived cell lines. (B) Bioinformatic analysis of drug responses associated to mutations in the drug efflux pump ABCB1 or in FBXW7. (C) OSEA analysis of the “Mitochondrial translation” hallmark, done with proteomics data from FBXW7+/+ and FBXW7−/− DLD-1 cells.

(b) Viability of FBXW7+/+ and FBXW7−/− DLD-1 cells exposed to increased doses of the antibiotic tigecycline at the indicated doses.

FIGURE 2

(A) Distribution of chromatin-bound SUMO2/3 and VCP upon USP7 inhibition in U2OS cells. Note the accumulation of VCP in areas that become enriched in SUMOylated factors. (B) Proteomics of the VCP-interactome upon inhibition of USP7 (P22) or VCP (NMS). The analyses revealed enrichment of the VCP adaption FAFA1 and FAP1 upon either form of inhibition, as well as a selective increase in the binding of VCP to SUMO2 upon USP7 inhibition.