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CRISPR screens and patient stratification

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The paper highlighted below contains data from a CRISPR–Cas9 knockout screen performed by scientists at Horizon Discovery. The screen was carried out in HT-29 colorectal cancer cells in the presence of an ATM inhibitor from AstraZeneca and the DNA topoisomerase 1 inhibitor irinotecan (SN-38), a drug combination that is currently part of a Phase 1 clinical trial. The data from the screen indicate potential routes for patient stratification.

A recent paper by Stephen Jackson, Josep Forment and colleagues used CRISPR–Cas9 knockout screens to examine the complexities of drug resistance in cells with compromised DNA damage response (DDR) pathways. Their data should enable the subtle differences that govern cellular responses to DNA damaging agents to be further exploited in the clinic.

The cellular mechanisms that have evolved to deal with DNA damage are intricate and require painstaking experiments to understand how each DNA damage pathway interacts and which cues prompt the use of different pathway components. Cancer cells exhibit vulnerabilities to specific drugs that impact DDR pathways: poly (ADP-ribose) polymerase inhibitors (PARPi) in BRCA-deficient cells being the clinical posterchild. BRCA-deficient cells have compromised homologous recombination repair (HRR) pathways, which are normally active during S-phase of the cell cycle. Treatment of BRCA-deficient cells with PARPi induces PARP trapping on DNA and the accumulation of unrepaired single-strand DNA breaks, lesions that are converted into more toxic double-strand DNA breaks (DSB) by active replication during S-phase. In the absence of HRR these DSBs are mis-repaired through the non-homologous end joining (NHEJ) repair pathway, which results in chromosomal aberrations and cell death.

Cell lines in which the ataxia telangiectasia mutated (ATM) gene is compromised show heightened sensitivities to a range of DNA-damage inducing therapies, including ionizing radiation (IR), DNA topoisomerase I (TOP1) poisons such as topotecan, and PARPi. Given this, the authors asked whether ATM and BRCA compromised cells share resistance mechanisms to agents that cause replication-induced DSBs.

To explore the mechanisms of resistance and sensitivity to topotecan in ATM deficient cells, the authors carried out a whole genome CRISPR–Cas9 knockout screen in *Atm* wild type (WT) and *Atm* null isogenic mouse embryonic stem cells (mESCs). Unsurprisingly, the top hit for drug resistance in the *Atm* WT mESCs was the drug target TOP1. However, in the *Atm* null mESCs, the NHEJ factors XRCC4 and LIG4 were the top hits, along with components of the BRCA1-A complex BRE, FAM175A and BABAM1, which negatively modulate HRR. CRISPR–Cas9 was used to engineer knockout of these hits in ATM deficient mESCs and human RPE-1 cells and resulted in resistance to topotecan, validating the identification of these genes as resistance targets in the initial CRISPR–Cas9 whole genome screen.

Are the resistance mechanisms identified in the mESCs relevant to the clinic? AstraZeneca is currently sponsoring a clinical trial in patients with colorectal cancer testing the combination of the TOP1 inhibitor irinotecan (SN-38) with the ATM inhibitor AZD0156 (NCT02588105). A CRISPR–Cas9 knockout screen in the colorectal cancer cell line HT-29 carried out in the presence of both inhibitors identified components of the NHEJ and BRCA1-A complexes as mediators of resistance to both agents. The top hit from this screen is BRE, followed closely by NHEJ1, FAM175A, LIG4 and XRCC4. These data indicate that in patients with ATM deficient tumours, or where an ATM inhibitor is being used, the presence of mutations that disrupt either NHEJ repair or the BRCA1-A complex might lead to resistance to a TOP1 inhibitor.

The additional extensive experiments in this paper indicate that the toxicity induced by topotecan in ATM deficient cells is likely the result of the formation of chromosomal aberrations. These most likely arise from an imbalance in the DNA repair pathways with HRR being slow to establish in ATM deficient cells allowing NHEJ to mis-repair single-ended DSBs produced at collapsed replication forks in S phase. Interestingly, the mechanisms in ATM deficient cells that lead to resistance to drugs that induce these single-ended DSBs are different to those evident in BRCA1 mutant cells, suggesting routes for defined patient stratification and new avenues for drug development.

Another interesting aspect of this paper is that it represents a highly successful collaboration between academic, industrial and contract research scientists. “It has been wonderful collaborating with the group of Prof Steve Jackson and with Horizon Discovery to carry out these exciting studies”, said Josep Forment, Team Leader, AstraZeneca Oncology. Benedict Cross, Head of Functional Genomic Screening at Horizon, concurred “The execution of these screens shows a perfect example of where an important research programme is lead collaboratively by industry and academia, and is a good example of where Horizon can leverage our industrialised screening platform to quickly deliver high quality data to contribute”. Joseph and his colleagues AstraZeneca are now “exploring how these findings might lead to the discovery of more effective cancer treatments”

Balmus, G. *et al.* ATM orchestrates the DNA-damage response to counter toxic non-homologous end-joining at broken replication forks. Nat. Comms. 10.1038/s41467-018-07729-2 (2019)

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