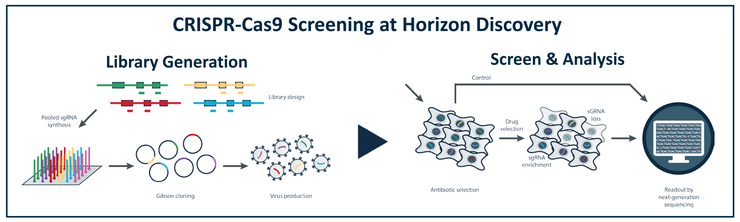
HORIZON DISCOVERY

CRISPR-Cas9 sensitivity and resistance screens in haploid and isogenic human cancer cell lines

Jessica R. Hunt, Steffen Lawo, David Walter, Isabelle Nett, Joanne Yarker, Paul Russell, Benedict C.S. Cross, Ceri M. Wiggins and Jonathan D. Moore

The discovery of the CRISPR-Cas system in bacteria has initiated an impressive array of innovations that have enabled the use of the RNA-guided Cas9 nuclease in functional genomic screens. At Horizon Discovery, we have embraced these developments, as they provide new opportunities for drug target identification and validation.



eHAP cells are a fully haploid cell line developed by Horizon Discovery, and these cells offer several advantages over conventional cancer cells for genetic screens. Screens in haploid cells maximise editing efficiency and consequently have a lower signal-to-noise ratio than screens using cells of higher ploidy, a significant advantage when studying sensitive biological paradigms. In addition, haploid cells can be used to rapidly evaluate hits as part of post-screen validation cascades, making them a valuable tool for drug discovery work-flows.

We have examined the power of haploid-based CRISPR-Cas9 screens for identifying genes that when knocked out increase sensitivity to a specific stimulus, such as a cancer therapeutic, compared with a vehicle control. The cells with increased sensitivity will be lost from or fail to expand in the cell population present at the end of the screen, thus the screen looks for the drop-out of sgRNAs to identify targets. These targets could represent novel synthetic lethal interactions that can be exploited by combination therapies.

Members of the kinesin family mediate sensitivity to paclitaxel

Following infection with the GeCKOv2 whole genome library, eHAP cells were cultured in the presence or absence of low dose paclitaxel, to identify genes whose loss resulted in an increased sensitivity to paclitaxel treatment. Paclitaxel is an antimitotic agent that irreversibly binds tubulin, giving rise to defects in mitotic spindle assembly, chromosome segregation and cell division. Interestingly, loss of several genes associated with the cytoskeleton were found to increase sensitivity to paclitaxel (Figure 1).

These included several kinesins, a family of motor proteins that move along microtubules to support normal cell division. MLST8, a component of the mTORC2 complex, also increased cell sensitivity to paclitaxel, possibly owing to its function upstream of Rho-GTPases that regulate the actin cytoskeleton. In addition, loss of the multidrug transporter ABCB1, which mediates the cellular efflux of paclitaxel, also increased cell sensitivity to this drug.

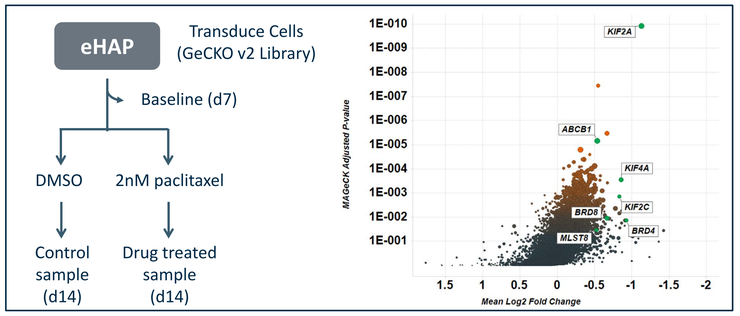


Figure 1: Paclitaxel sensitivity screen in eHAP cells
Ranking of screen hits by the MAGeCK hit calling algorithm, identifying genes that when lost sensitise eHAP cells to paclitaxel treatment.

Targets identified in the pooled CRISPR-Cas9 screen were validated using pre-existing stocks of HAP1 knock-out cell lines that are readily available from Horizon Discovery. Using this approach, we were able to rapidly validate and exclude several kinesins as genes whose loss conferred paclitaxel sensitivity. The *ABCB1* and *BRD8* genes were also validated using this approach (Figure 2).

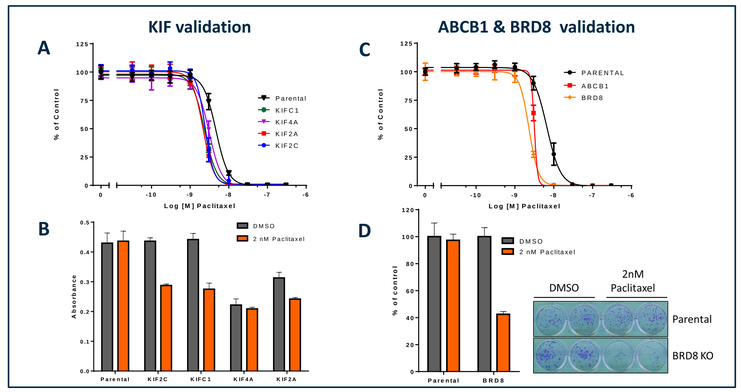


Figure 2: Target validation using HAP1 knock-out cell lines
HAP1 KO clones generated using CRISPR-Cas9 gene editing were used to validate the potential hits. Parental and KO cells were assessed for paclitaxel sensitivity by 72h proliferation assay (Figures 2A and 2C) using ATPlite to measure cell growth. Clones were also assessed in a 7 day 2D colony formation assay (Figures 2B and 2D), where resulting colonies were fixed, stained with crystal violet and solubilised to allow quantification.

Haploid cells are a responsive model for CRISPR-Cas9 sensitivity and resistance screens

We have also used the eHAP cells and GeCKOv2 whole genome library to identify factors that increase cell sensitivity to glucose-depleted conditions. Use of an adjusted p-value for gene ranking (Li *et al.*, 2015) identified several NADH-dehydrogenase enzymes and two tumour suppressors (*TSC1* and *TSC2*) as potential hits (Figure 3). As expected, oxidative phosphorylation and electron transport chain encoding genes were also identified.

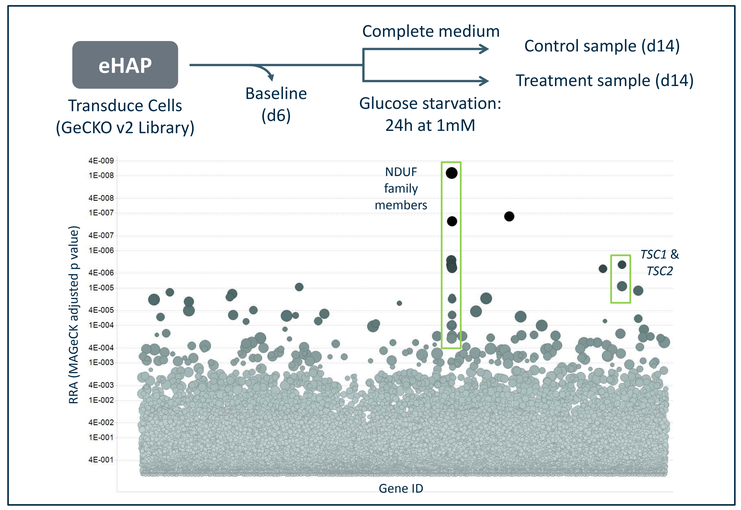


Figure 3: Glucose Starvation sensitivity screen in eHAP cells
Ranking of screen hits by the MAGeCK hit calling algorithm, identifying genes that when lost sensitise eHAP cells to glucose starvation.

A genome-wide positive selection screen in eHAP cells was carried out to identify genes whose loss of expression induces resistance to the purine antimetabolite 6 thioguanine (6-TG). NGS analysis identified *MLH1*, *MSH2* and *MSH6* as targets, genes known to be involved in resistance to 6-TG (Figure 4A). These targets were validated as resistance factors using HAP1 knockout cells, all of which showed reduced sensitivity to 6-TG compared to the parental cell line (Figure 4B).

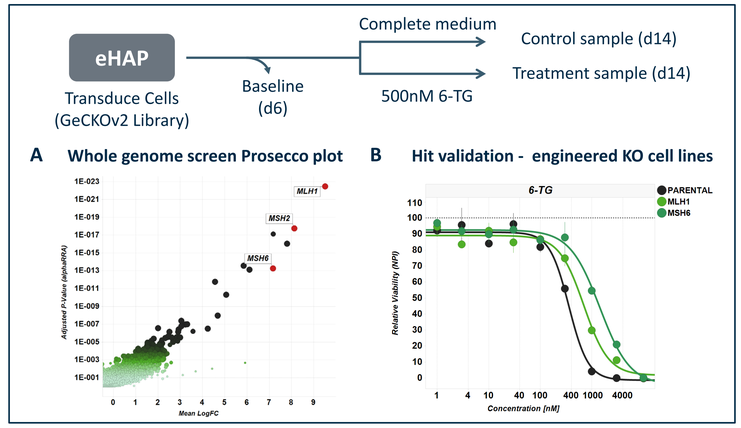


Figure 4: Identification and validation of 6-TG resistance factors in HAP1 cells. (A) Ranking of screen hits by the MAGeCK hit calling algorithm. On the y-axis, genes are ranked by robust ranking aggregation values for their enrichment after 6-TG treatment. The mean log2-fold change in sgRNAs targeting the same gene are plotted on the x-axis. (B) *MLH1* and *MSH6* knockout HAP1 cell lines were seeded and treated with increasing concentrations of 6-TG, and the affect on cell proliferation assessed.

Synthetic lethal CRISPR-Cas9 screens in isogenic cells

We have also used Horizon Discovery's DLD1 PI3K isogenic cell lines to identify targets that show selective lethality in the presence of the activating *PIK3CA*^{E545K} mutation. Using a custom made sgRNA library based on the druggable genome, we identified several potential synthetic lethal interactions in *PIK3CA*$^{E545K/+}$ cells including *PIK3CA* and the downstream kinases *AKT1* and *AKT2.* A requirement for PI3K signalling in DLD1 isogenic cells that contain only a single wildtype *PIK3CA* allele was also found, but in this case the cells are reliant on the upstream stimuli through *EGFR*, which is dispensable in *PIK3CA*$^{E545K/+}$ cells (Figure 5).

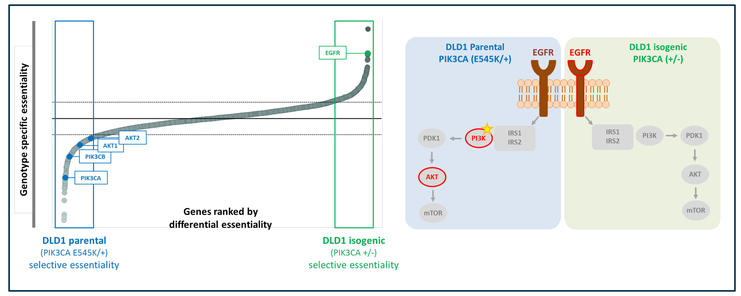


Figure 5: Comparison of gene essentiality in DLD1 parental (PIK3CA E545K/+) and isogenic (PIK3CA +/-) cells
To identify PIK3CA-essential genes, the log2 sgRNA fold change distribution of each line was mean-normalised to zero. For each sgRNA, a differential essentiality score was then defined as the average log2 fold change in the DLD1 PIK3CA E545K/+ parental line subtracted by the average log2 fold change in the PI3KCA +/- isogenic line.

Conclusion

The application of CRISPR-Cas9 technology to whole genome screens is revolutionising our ability to perform target identification experiments and rapidly validate hits. In particular, haploid cells provide a genetically clean, sensitive system for screening that may facilitate the identification of more subtle biological interactions.

t + 44 (0)1223 655580
f + 44 (0)1223 655581
e info@horizondiscovery.com
w www.horizondiscovery.com
Horizon Discovery, 7100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom