Introduction

Pooled CRISPR–Cas9 knock out screens provide a valuable addition to the methods available for novel drug target identification and validation. However, where gene editing is targeted to amplified loci, the resulting multiple DNA cleavage events can be a cause of false positive hit identification. The generation of nuclease deficient versions of Cas9 has enabled the generation of two additional techniques – CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) – that enable the repression or overexpression, respectively, of target genes.

Here we report the first direct combination of all three approaches (CRISPRko, CRISPRi and CRISPRa) in the context of genome-wide screens to identify components that influence resistance and sensitivity to the standard of care BRAF inhibitor, vemurafenib.

Drug-gene interaction screening

As a comparison to our CRISPRi screen, we also evaluated a CRISPRko approach side-by-side. Both loss-of-function tools yielded a series of validated and novel vemurafenib resistance hits, but the CRISPRi system showed substantially greater sensitivity. The increased sensitivity is in part a consequence of the adapted tracrRNA this screen benefited from (Cross et al., 2016) but also demonstrates the quality and precision of the screening technology. Individual guide analysis for the CRISPRi screen demonstrated a high performance of the library. A number of novel hits found only by the CRISPRi screen showed a strong degree of essentiality, supporting the value of this tool in finding hits of this classification.

In the CRISPRi screen, vemurafenib resistance was primarily conferred by an increased activation of genes involved in receptor tyrosine kinase (RTK), G-protein coupled receptor and integrin (ITG) signalling pathways. Activation of these pathways aids in bypassing inhibited BRAF kinase signalling, which is in concordance with data published previously (Konermann et al., 2015).

Systematic hit ID by dual-direction screening

Combination and comparison of the gain- and loss-of-function screening datasets allowed the interrogation of the opposing effects and evaluation of whole gene networks. Components within these networks could respond variably to either activation or inhibition based on their respective biological role in the targeted pathway.

Summary and conclusions

Our data indicate that CRISPRi and CRISPRa are valuable additional new CRISPR screening tools for target identification and validation. Importantly, with appropriate design, the power of enrichment-based screening (e.g. resistance screening) can now be co-opted to identify genes which result in sensitisation by analysing the effect of the opposing function. Whilst the effect of depletion of a target gene on loss of cell viability might be hard to study with loss-of-function screen, the response of cells to hyper-activating this component on overcoming cell death can be readily detected, providing valuable genetic insights into cellular physiology.