Dual CRISPRi and CRISPRa screening reveals phenotypic switches in BRAF inhibition

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Introduction

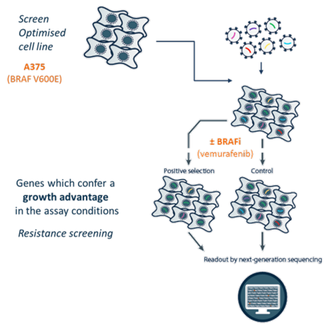
Pooled CRISPR–Cas9 knock out screens provide a valuable addition to the methods available for novel drug target identification and validation. However, where gene editing is targeted to amplified loci, the resulting multiple DNA cleavage events can be a cause of false positive hit identification. The 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.

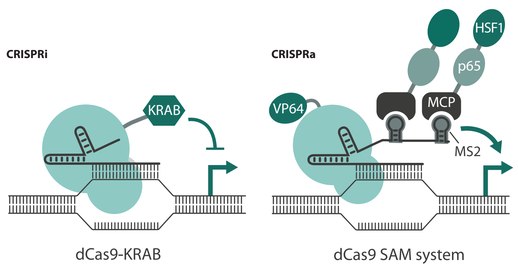
Platform design, development and validation

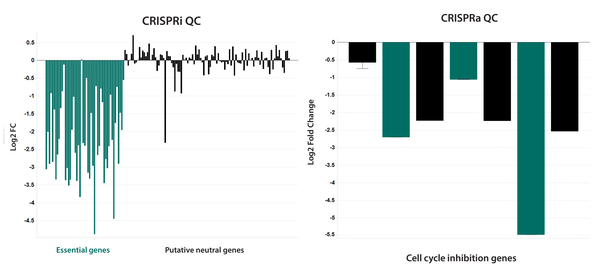
For CRISPRi screening we produced a custom all-in-one lentiviral vector system containing a dCas9-KRAB fusion as well as a U6-promoter driven guideRNA cassette. The simultaneous expression of both dCas9-KRAB and CRISPR from a single lentiviral insert provides a highly efficient targeting strategy required for pooled functional screening. To further enhance performance, we implemented our modified tracrRNA (Cross *et al*., 2016). For CRISPRa screening we used the synergistic activation mediator (SAM; Konermann *et al*., 2015), comprising a combination of dCas9-VP64, MS2-p65-HSF1 and U6-promoter driven guideRNA. We constructed two human whole-genome guide RNA libraries based on the latest guide RNA design by Horlbeck *et al.* (2016) who have demonstrated strongly improved performance of guide RNAs that bind to genomic DNA locations away from local nucleosomes.

To test our platform we designed a proof-of-concept screen in which we evaluated the genetic modulations which would lead to resistance to the BRAF inhibitor, vemurafenib. A375 melanoma cells carry a BRAF V600E gain-of-function mutation and we hypothesized that transcriptional modulation with CRISPRa and CRISPRi would allow a subset of cells in the population to become resistant to vemurafenib treatment as a result of altered gene transcription. These would be detected by their increased abundance by deep sequencing.



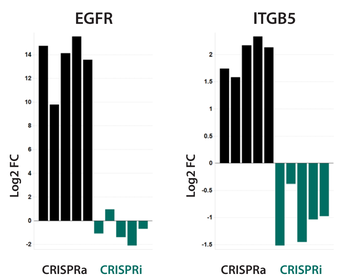
For both platforms we compared guide RNA abundance in control cells to the library input. In the case of CRISPRi, we observed substantial drop-out of key essential genes from the cell population when targeted for repression, as expected. This confirmed the quality of the screening tools and demonstrated excellent sensitivity and precision. In the case of CRISPRa, we observed genes mainly involved in negatively regulating the cell cycle to drop out significantly. These results are consistent with the expected response to the hyper-activation of genes that control the blockade of cell division and provide a valuable quality control measurement for the ability of this platform to generate locus-specific gene expression amplification.

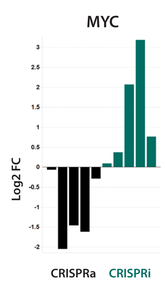




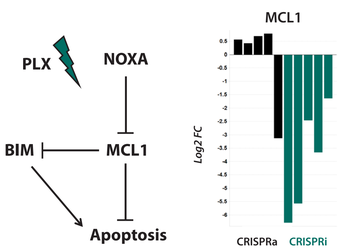
Systematic hit ID by dual-direction screening

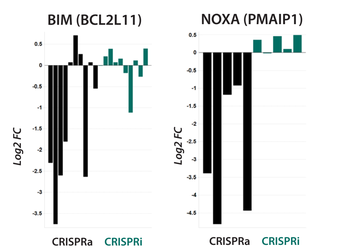
Combination and comparison of the gain- and loss-of-function screening datasets allowed the interrogation of the opposing effects and evaluation of whole gene networks. Components within these networks could respond variably to either activation or inhibition based on their respective biological role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





In addition to providing a compelling strategy for the validation of drug resistance targets, the parallel analysis of CRISPRi and CRISPRa data sets also allows the identification of pathway modulators that affect both drug sensitivity and resistance, but in which sgRNAs are not reciprocally lost or increased in each screen. This was particularly evident when we examined the triggers to apoptosis on vemurafenib and the controlling components MCL1, BIM and NOXA.



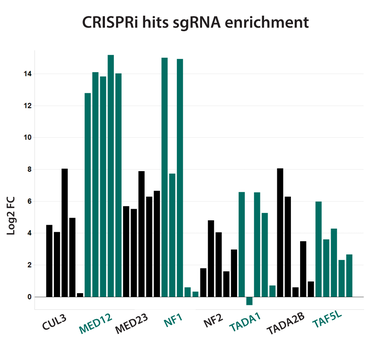


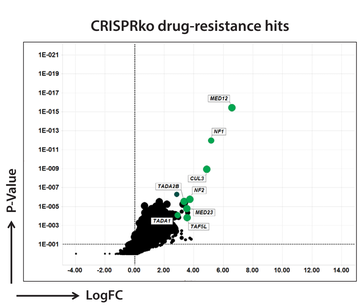
Drug-gene interaction screening

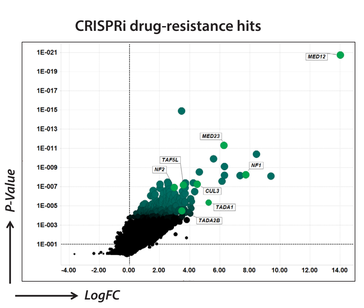
As a comparison to our CRISPRi screen, we also evaluated a CRISPRko approach side-by-side. Both loss-of-function tools yielded a series of validated and novel vemurafenib resistance hits, but the CRISPRi system showed substantially greater sensitivity.

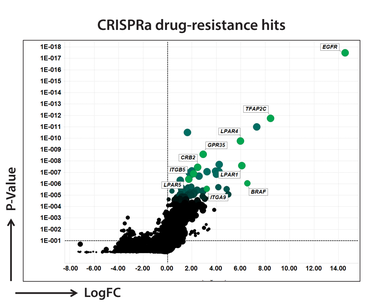
The increased sensitivity is in part a consequence of the adapted tracrRNA this screen benefited from (Cross *et al*., 2016) but also demonstrates the quality and precision of the screening technology. Individual guide analysis for the CRISPRi screen demonstrated a high performance of the library. A 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.

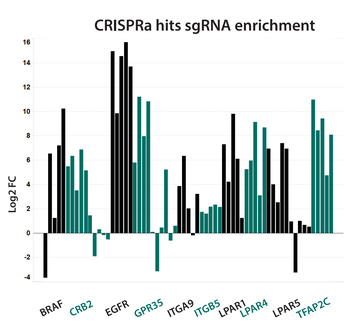
In the CRISPRa screen, vemurafenib resistance was primarily conferred by an increased activation of genes involved in receptor tyrosine kinase (RTK), G-protein coupled receptor and integrin (ITG) signalling pathways. Activation of these pathways aid in bypassing inhibited BRAF kinase signalling, which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











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.

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