APPLICATION NOTE

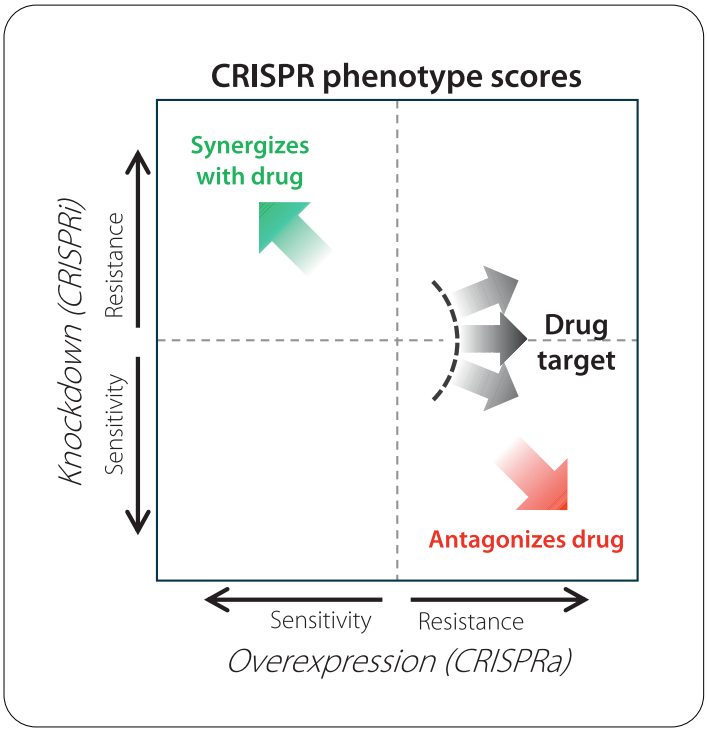
The dual screen: Parallel genome-wide CRISPRi & CRISPRa screening.

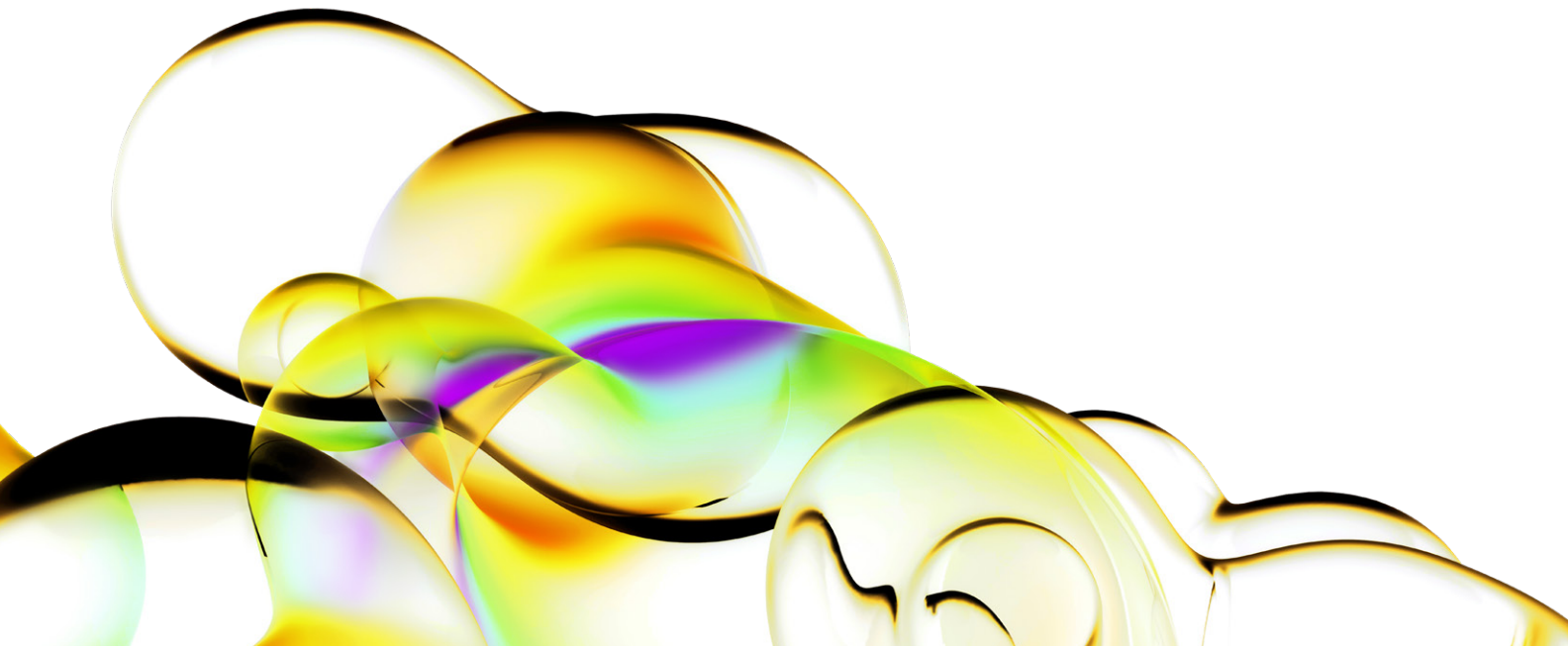
Authors

Carlos le Sage
Benedict CS Cross

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.



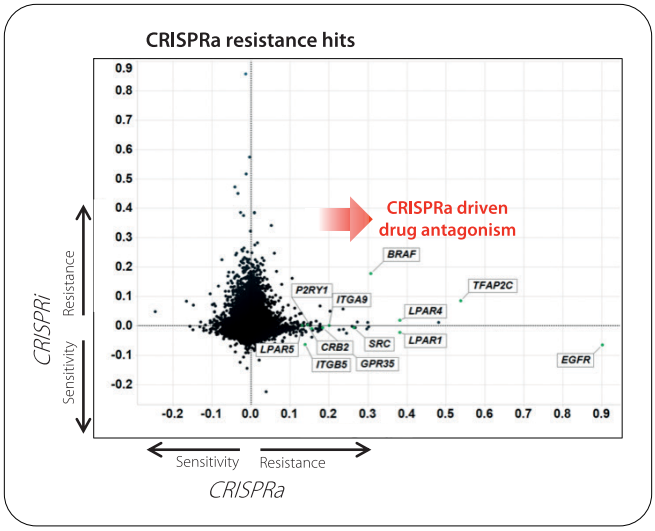


The dual screen uses CRISPRi and CRISPRa gene modulation technologies in parallel and provides the ability to identify drug-gene interactions through paired gene perturbation analysis. The approach offers researchers a novel, powerful and systematic way to explore drug mechanisms of action, enables the identification of novel bio-markers as well as providing compelling targets of use for the development of combination therapies.

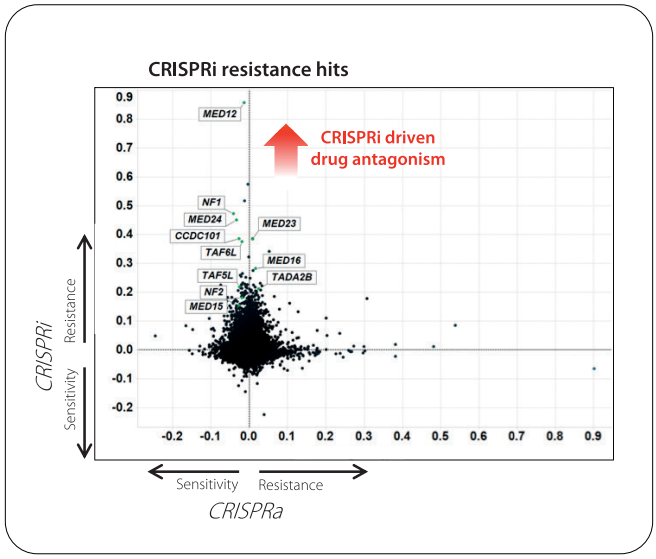
Proof of concept screening

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 (Le Sage et al., 2017). A375 melanoma cells carry a BRAF V600E gain-of-function mutation, therefore 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 cells would be detected by their increased guideRNA abundance, as analysed by deep sequencing.

Systematic hit ID by dual-direction screening reveals vemurafenib resistance mechanisms

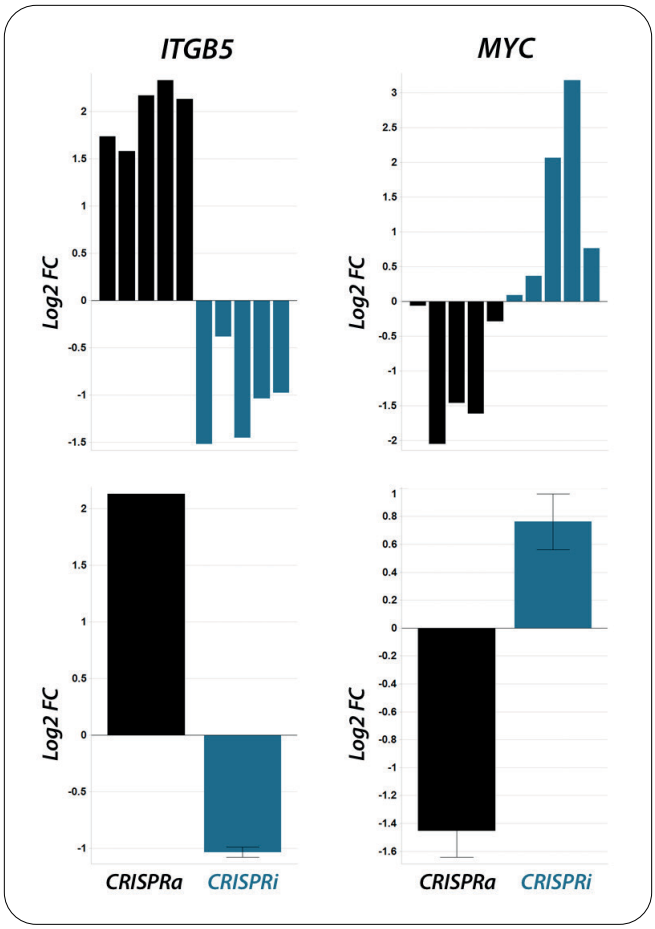


In the CRISPRa screen, vemurafenib resistance was primarily conferred by an increased activation of genes involved in receptor tyrosine kinase, G-protein coupled receptor and integrin (ITG) signalling pathways. Activation of these pathways aid in bypassing inhibited BRAF kinase signalling, which is in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



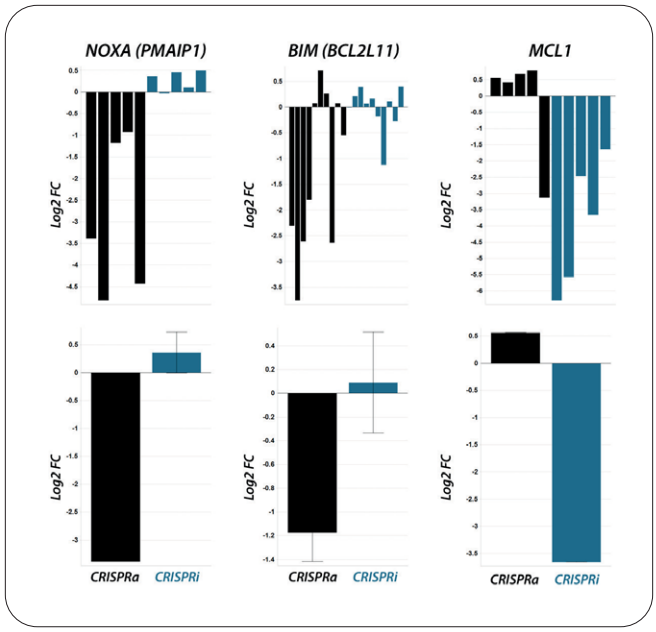
CRISPRi loss-of-function screening yielded a series of validated and novel vemurafenib resistance hits at great sensitivity, which in part is a consequence of the adapted tracrRNA this screen benefited from, (Cross et al., 2016) but also demonstrates the quality and precision of this screening technology. 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 in the CRISPRko approach, supporting the value of this tool in finding hits of this classification (Le Sage et al., 2017).

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. This is exemplified by MYC, which sensitizes cells to vemurafenib in the CRISPRa screen, while resisting treatment in CRISPRi, and ITGB5, whose gene modulation shows the opposite pattern.



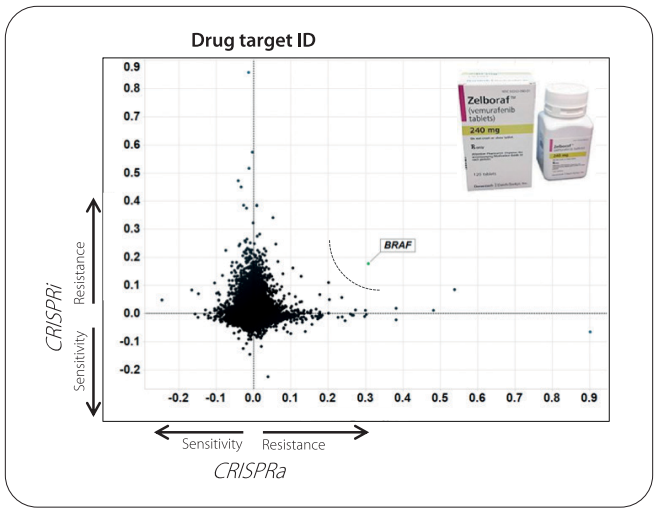
CRISPR dual screen reveals unique drug response signature

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 guideRNAs 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. Importantly, the dual screen approach therefore allows researchers to maximise hit ID within pathways and substantially improves the likelihood for high fidelity target discovery. Taking each screen independently provides valuable functional genomic information, but only by combining the datasets is a more complete discovery opportunity realised.



Dual-direction screening identifies BRAF as vemurafenib target

Finally, the use of dual genome modulation screening led to the direct and unambiguous identification of the molecular target of vemurafenib, BRAF. The BRAF gene uniquely resists drug treatment when overexpressed by CRISPRa, but also when knocked down in the CRISPRi screen. The latter has been proposed to depend on the paradoxical activation of MEK/ERK by vemurafenib-bound CRAF homodimers (Poulikakos et al., 2010).



Summary and conclusions

Our data indicates that the dual screen approach is a crucial new screening tool for target identification and validation. This combination approach is of particular value since with the appropriate design, the power of enrichment-based screening (*e.g.* resistance screening) can now be exploited to identify genes which result in sensitization by analyzing the effect of the opposing function. The effect of depletion of a target gene on cell viability might be hard to study with loss-off-function screening, but with the dual screen the response of cells to hyper-activating this component to overcome cell death can be readily detected, providing valuable genetic insights into cellular physiology.

Methods

Dual direction screening was performed in A375 melanoma cells. To enable LOF screening, cells were infected with Revvity's CRISPRi-dCas9 lentivirus at low MOI. For GOF screening, A375 cells were transduced with dCas9-VP64 lentivirus followed by MS2-p65-HSF1 lentivirus, both at high MOI to ensure higher copy-number per cell.

Finally, a CRISPRa library lentivirus was introduced, at low MOI, to ensure integration of a single construct per cell, similar to CRISPRi. Cells carrying integrated lentivirus were selected with an antibiotic to eliminate non-transduced cells. The screen dose phase was then initiated and cell populations were treated with media containing vemurafenib (2μM) or control (DMSO), each replicated twice. When control treated cells reached population doubling 16, end pellets were collected, genomic DNA was extracted and guide RNA abundance in each condition measured by NGS. Phenotype scores for each guideRNA for both screens was calculated using the following formula: Log2 Fold change/ (PD^{CTRL} – PD^{VEM}), where PD represents population doublings.

References

Cross, B. C. S. et al., Sci. Rep. 2016; 6(31782)

Jost, M. et al., Mol. Cell. 2017; 68

Konermann, S. et al., Nature. 2015; 517(7536)

Le Sage, C. et al., Sci. Rep. 2017; 7(17693)

Poulikakos, P.I. et al., Nature. 2010; 464(7287)

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA

(800) 762-4000
www.revvity.com