CRISPRa: Transcriptional upregulation screening with genome-wide CRISPR activation.

Authors

Carlos le Sage
Benedict CS Cross

Revvity, Inc.

Introduction

Building on the transformative impact of pooled screening using CRISPR-Cas9, researchers have developed catalytically inactive Cas9 (dCas9) and have demonstrated that it can be used to tune genetic output. These systems are known as CRISPRi (interference) and CRISPRa (activation), respectively.

Revvity have developed both human whole-genome CRISPR-Cas9 knockout and CRISPRi-dCas9 platforms and have demonstrated the power of both systems in identifying resistance genes to the BRAF inhibitor vemurafenib through loss-of-function screening.

We now present a human whole-genome CRISPRa platform and demonstrate its effectiveness in identifying resistance genes complementary to those identified through loss-of-function screening, under vemurafenib cytostatic conditions. The ability to enhance transcription by several orders of magnitude opens new avenues and for the first time offers researchers the opportunity to explore resistance mechanisms based on a CRISPRa-driven gain-of-function platform.

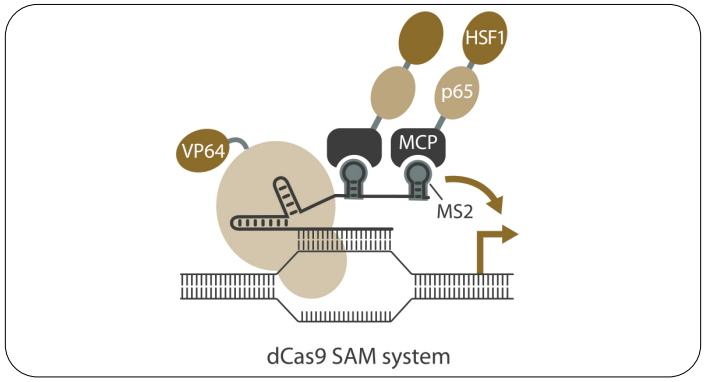
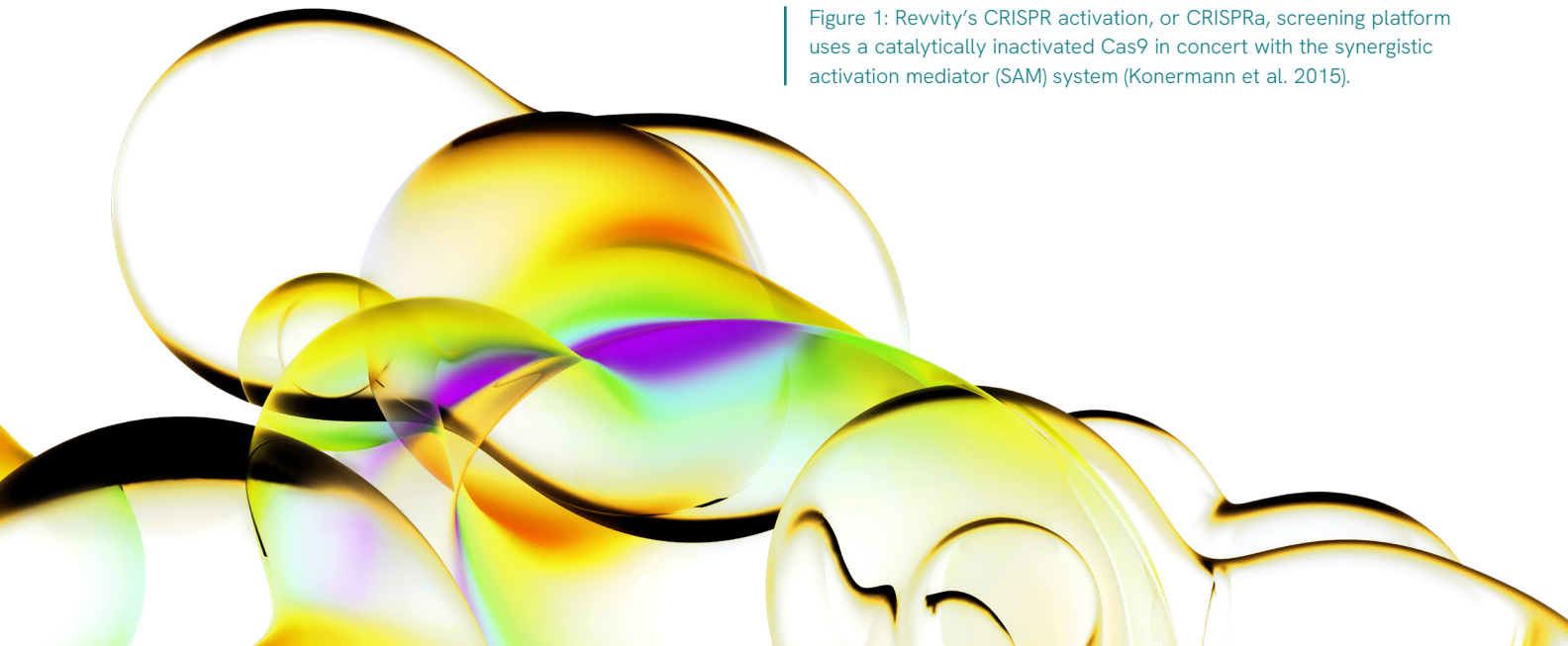


Figure 1: Revvity's CRISPR activation, or CRISPRa, screening platform uses a catalytically inactivated Cas9 in concert with the synergistic activation mediator (SAM) system (Konermann et al. 2015).



Platform design

For CRISPRa screening we used the synergistic activation mediator (SAM) system as previously described by the Feng Zhang lab (Konermann et al., 2015). This system is a three-component platform consisting of lentiviral vectors dCas9-VP64, MS2-p65-HSF1 and U6-promoter driven guideRNA cassette with a tracrRNA modified to facilitate binding to MS2-p65-HSF1 activating modules. We constructed a human whole-genome guideRNA library based on the latest improved guideRNA design from Horlbeck et al (2016). This second generation hCRISPRa library contains 5 guide RNAs per gene targeting a total of 19,050 genes.

Proof-of concept screening

As proof of concept we performed a genome-wide positive selection screen to identify genes associated with vemurafenib resistance (PLX-4032). Vemurafenib is a BRAF kinase inhibitor which has been shown to act cytostatically in A375 melanoma cells, which carry a BRAF V600E gain-of-function mutation (Davies et al., 2002; Flaherty et al., 2010; Shalem et al., 2014).

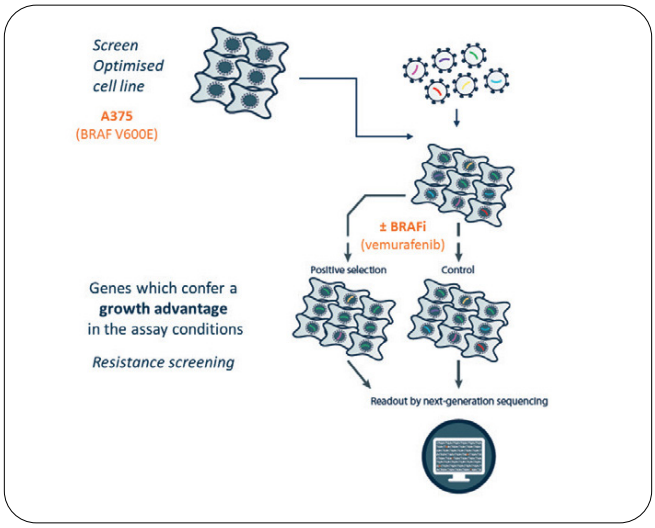


Figure 2: Screen schematic. Resistance to vemurafenib was monitored in response to CRISPRa screening to evaluate the platform performance.

After 16 days of vemurafenib treatment, screen end pellets were taken and the relative abundance of each of the guide RNAs was measured by next-generation sequencing.

CRISPRa screening platform validation

First we compared guide RNA abundance in DMSO control treated cells to the plasmid library input, and 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.

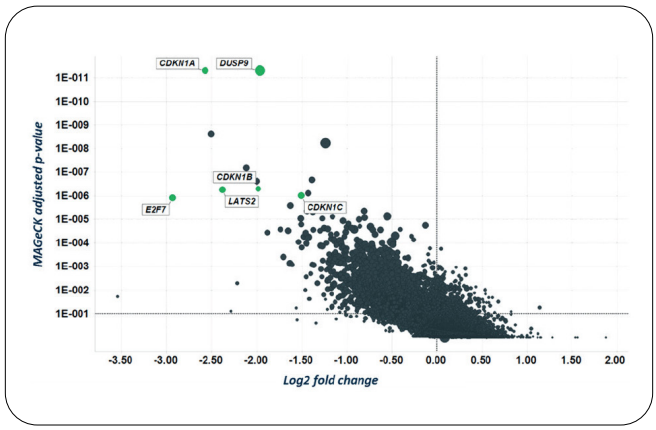


Figure 3: Genes which selectively drop-out over the course of the CRISPRa screen. A collection of cell cycle inhibitors were identified, which when over-expressed would be expected to cause a detrimental effect on the survival of the cells, and therefore “drop-out” of the screen.

CRISPRa screening hit identification

Gene-level hit identification was conducted using assimilated scores from each of the guides targeting each gene. 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).

Moreover, pathway members of each of these mechanisms which had not been previously identified by genome-wide screening were identified by our proof-of-concept screen.

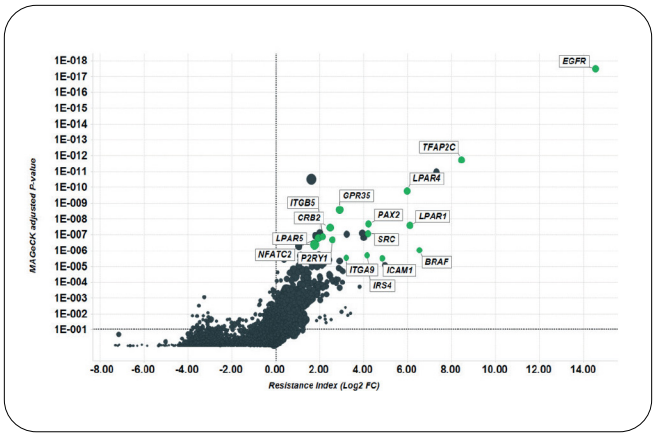


Figure 4: Data from CRISPRa vemurafenib screening analysis showing log2 fold enrichment of each gene and associated p-values. Highlighted hits have been previously identified and validated by CRISPRa screening with an older generation library (Konermann et al., 2015).

Importantly, evaluation of the individual guide RNAs of the selected resistance top hits demonstrated that only a few guide RNAs did not perform optimally. Overall this indicates the robustness of CRISPRa and reflects its ability to effectively upregulate gene expression leading to resistance to vemurafenib.

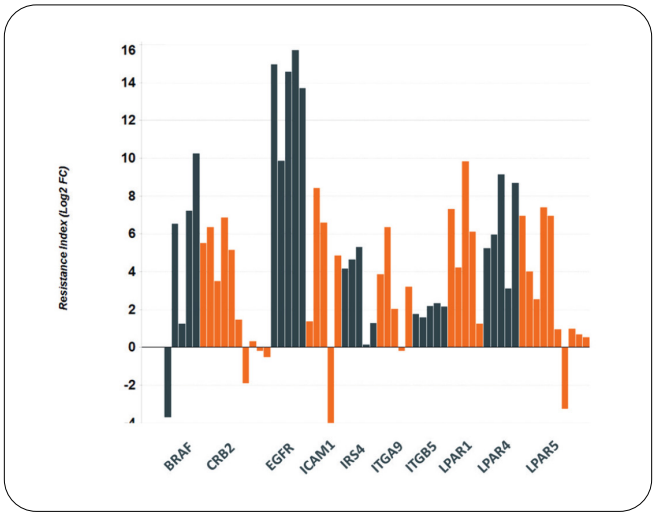


Figure 5: Data from CRISPRa vemurafenib screening analysis showing log2 fold enrichment of each of the guides targeting each of the selected gene-level hits.

Comparison of our novel dataset with previously conducted screens evaluating the same experimental paradigm reveal an outstanding level of overlap, with all genes identified by Konermann et al (2015) also identified in our screen. In general, the robustness and magnitude of each hit is amplified using this new platform compared (Horlbeck et al., 2016).

Moreover, our screen identified a great number of additional hits which either fell into the same genetic pathways as validated hits, or represent novel opportunities for therapeutic development.

Table 1: Hit ID table showing previously identified hits in green and novel genes (anonymised; GENE) for future validation.
* are genes identified as sensitivity hits in CRISPRi.

GENE	RRA score	p-value	FDR	Rank	LogFC
EGFR*	3.11E-18	2.64E-07	0.000825	1	14.097
TFAP2C	2.47E-12	2.64E-07	0.000825	2	8.4113
GENE	1.32E-11	2.64E-07	0.000825	3	7.5401
GENE	8.51E-11	2.64E-07	0.000825	4	1.385
LPAR4	1.25E-10	2.64E-07	0.000825	5	5.9706
GPR35	2.29E-09	2.64E-07	0.000825	6	2.8763
GENE	2.05E-08	7.93E-07	0.00165	7	4.1775
LPAR1	2.63E-08	7.93E-07	0.00165	8	5.9617
CRB2	2.79E-08	7.93E-07	0.00165	9	2.2622
GENE	6.79E-08	1.32E-06	0.002166	10	1.8138
SRC	1.05E-07	1.59E-06	0.002166	11	4.1304
GENE	1.34E-07	1.85E-06	0.002166	12	3.7063
GENE	1.36E-07	1.85E-06	0.002166	13	2.2585
ITGB5*	1.38E-07	1.85E-06	0.002166	14	2.1651
LPAR5	1.49E-07	1.85E-06	0.002166	15	2.0537
GENE	1.61E-07	1.85E-06	0.002166	16	3.8346
P2RY1	2.43E-07	2.38E-06	0.002621	17	2.4375
GENE	2.66E-07	2.64E-06	0.00275	18	1.7958
GENE	5.12E-07	2.91E-06	0.002866	19	3.1365
GENE	7.70E-07	3.44E-06	0.003218	20	1.4929
GENE	1.17E-06	7.67E-06	0.006836	21	1.1072
ITGA9	1.57E-06	9.78E-06	0.007807	22	3.1412
GENE	1.66E-06	1.03E-05	0.007807	23	1.7808
GENE	1.68E-06	1.03E-05	0.007807	24	1.4059
RAPGEF6	1.71E-06	1.08E-05	0.007807	25	1.9992

Summary

We have used a proof-of-concept approach to examine the ability of our new CRISPRa-dCas9 platform to produce resistance to the BRAF inhibitor vemurafenib. We were able to identify all validated genes previously shown to confer resistance to vemurafenib treatment.

This new CRISPRa platform will now allow us to study gain-of-function phenotypes, as well as to complement CRISPRi and CRISPR-KO based functional genomic screening by evaluating gene networks affected by both loss- and gain-of-function analysis.

Methods

A375 melanoma cells were infected with dCas9-VP64 lentivirus followed by MS2-p65-HSF1 lentivirus, both at high MOI to ensure higher copy-number per cell. Finally, CRISPRa library lentivirus was introduced, at low MOI, to ensure integration of a single construct per cell. 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 in two replicates. 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.

References

Davies et al., Nature. 2002 Jun 27;417(6892):949-54

Flaherty et al., N. Engl. J. Med. 2010 Aug 26;363(9):809-19

Horlbeck, M. A. et al., eLife. 2016; 5

Konermann, S. et al., Nature. 2015;517(7536):583-8

Shalem et al., Science. 2014 Jan 3;343(6166):84-7

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com
Copyright ©2023, Revvity, Inc. All rights reserved.