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GENETIC SCREENS

CRISPR-based mapping of genetic interactions

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We often conceptualize genes as independent units of information, although their behaviour is influenced by interactions with other genes. Now, two independent studies present scalable double-knockout CRISPR-based screens for mapping pairwise genetic interactions and apply these to the identification of effective synergistic drug combinations in cancer.

Genetic interactions can be harnessed for clinical purposes, as seen for PARP inhibitors in cancer therapy, which exploit the synthetic lethality that arises from mutations in *BRCA1*. However, to fully utilize and understand pairwise genetic interactions, it is important to accurately map them genome-wide.

Writing in *Nature Biotechnology*, Han *et al.* present a scalable CRISPR–Cas9-based system for the high-throughput generation of double gene knockouts. The system was designed for easy cloning, multiplexing and compatibility with paired-end deep sequencing. To this end, the authors pooled two CRISPR libraries in which the lentiviral expression of 700 single guide RNAs (sgRNAs), targeting 207 druggable genes, was driven by either the human or mouse U6 promoter. The pooled library comprised ~490,000 paired sgRNAs corresponding to 21,321 drug target gene pairs.

Using a Cas9-expressing human immortalized cancer cell line (K562), the team produced a comprehensive map of genetic interactions on the basis of calculated scores. The genetic interaction score takes into account the deviation of an observed double-knockout phenotype from that expected on the basis of the corresponding two single knockouts.

The group's screening approach identified rare genetic interactions, including several that would be readily explained by known biological functions; for example, the genes *AKT1* and *AKT2*, which encode functionally redundant kinases, and the genes *BCL2L1* and *MCL1*, the protein products of which both function in apoptosis. Predicted gene pairs could be translated into synergistic drug combinations as exemplified by combination treatment using BCL2L1 and MCL1 inhibitors, which had a synergistic effect in chronic and acute myeloid leukaemia cells.

In *Nature Methods*, Shen *et al.* describe a dual sgRNA library for investigating pairwise genetic interactions. Constructs expressed two separate sgRNAs, with each designed to target one of two different genes. "Unlike previous studies, we sequenced sgRNAs over a time course," explains corresponding author Trey Ideker (University of California, San Diego). "The resulting growth curves proved more accurate than sequencing the endpoint of the experiment, an advance that may have practical implications for many CRISPR studies."

The authors screened three cell lines — HeLa, A549 and HEK293T cells — for pairwise combinations of tumour-suppressor genes and cancer-relevant drug targets. They identified 'hub' genes with multiple genetic interactions and were also able to compare and contrast the three cell lines, finding single-gene effects that recapitulated known biological differences between the lines. For example, a positive growth effect in A549 cells when *TP53* was knocked out was not observed in HeLa or HEK293T, in which *TP53* is already inactivated by viral proteins. Additionally, the team identified cell line-specific pairwise genetic interactions; intriguingly, interaction pairs were typically not shared between cell types. Identified interactions were tested and validated by targeting the gene pairs with small-molecule inhibitors in drug–drug assays.

The two related approaches demonstrate that CRISPR-based screens of gene interaction pairs can be leveraged to identify potential new drug targets for synthetic lethality. In addition, fine-mapping of genetic interactions can help to elucidate the genetic architecture of a cell line, pinpointing differences between cell types and how gene pairs can influence health and disease. This may help to personalize cancer treatment and enhance our understanding of cell and tissue development.

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ORIGINAL ARTICLES Han, K. *et al.* Synergistic drug combinations for cancer identified in a CRISPR screen for pairwise genetic interactions. *Nat. Biotechnol.* http://dx.doi.org/10.1038/nbt.3834 (2017) | Shen, J. P. *et al.* Combinatorial CRISPR–Cas9 screens for *de novo* mapping of genetic interactions. *Nat. Meth.* http://dx.doi.org/10.1038/nmeth.4225 (2017)