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Genetic Interaction Networks in Cancer Cells

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Abstract

The genotype-to-phenotype relationship in health and disease is complex and influenced by both an individual's environment and their unique genome. Personal genetic variants can modulate gene function to generate a phenotype either through a single gene effect or through genetic interactions involving two or more genes. The relevance of genetic interactions to disease phenotypes has been particularly clear in cancer research, where an extreme genetic interaction, synthetic lethality, has been exploited as a therapeutic strategy. The obvious benefits of unmasking genetic background-specific vulnerabilities, coupled with the power of systematic genome editing, have fueled efforts to translate genetic interaction mapping from model organisms to human cells. Here, we review recent developments in genetic interaction mapping, with a focus on CRISPR-based genome editing technologies and cancer.

INTRODUCTION

Our current knowledge of cancer cell function coupled with growing catalogues of genome sequence data for human tumours and cancer cells lines provides a rich foundation for precision oncology [e.g. The Cancer Genome Atlas (TCGA): https://cancergenome.nih.gov/; Catalogue of Somatic Mutations in Cancer (COSMIC) https://cancer.sanger.ac.uk/cosmic]. Genetically tailored therapeutics that take advantage of specific driver pathways can be designed to selectively kill cancer cells. For example, Trastuzumab, an antibody therapeutic that targets the HER2 receptor, is specific for HER2-positive breast cancers, while Imatinib, a tyrosine kinase inhibitor, targets the BCR-ABL fusion protein that drives most chronic myelogenous leukemias. Alternatively, therapies have been designed to exploit vulnerabilities generated by cancer cell-specific genetic variation. For example, BRCA1 and BRCA2-mutant breast and ovarian cancer cells are defective for DNA double strand break (DSB) repair, which renders the cancer cells hypersensitive to small molecules that inhibit the poly(ADP-ribose) polymerase 1 and 2 (PARP1/2) enzymes, which would otherwise initiate DSB repair through alternative mechanisms [1]. These examples illustrate the importance of understanding functional cancer genetics, yet current successes in the clinic that reflect decades of basic research have focused largely on a few key biological pathways. Clearly, there is much to be learned from unbiased systematic analyses of human gene function and genetic interactions (GIs), with a focus on unbiased identification of all

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biological pathways relevant to cancer cell division and discovering genetic variation that might be exploited to develop targeted therapeutics. In this article, we provide an overview of recent developments in the field of GI mapping with a particular focus on cancer.

CONTEXT-DEPENDENT GENE ESSENTIALITY AND THE CANCER PHENOTYPE

Recent improvements in genome editing technologies, most notably CRISPR (clustered regularly interspaced short palindromic repeats)-based methods, have accelerated the development of resources for genome-scale perturbation of genes in mammalian genomes, opening the door to systematic functional genomics analysis. The first and most widely used CRISPR technology pairs the Cas9 endonuclease with a gRNA (guide RNA) to target it to a given genomic site, where Cas9 induces a DNA double-strand break. Repair by non-homologous end-joining frequently results in insertions or deletions, leading to a functional knock-out of a gene of interest (Table 1). So far, efforts have largely focused on assessing the impact of individual gene perturbation on cell proliferation, a phenotypic read-out that reports on general cell physiology, is scalable and quantitative.

Libraries of both RNAi (RNA interference) knock-down reagents and gRNAs for genome-scale CRISPR gene targeting have been applied to human cell lines to identify essential genes required for cell proliferation. These studies revealed a core set of essential genes required for viability in most cell lines, including highly conserved genes whose functions are maintained from yeast to humans, as well as genes that are required for viability only in a subset of cancer cell lines (reviewed in [2,3]). Mirroring the general findings from studies of gene deletion mutants in yeast, the core human cell essential gene set only includes a relatively small fraction (~10%) of the genes in the human genome, highlighting the extensive functional buffering inherent to eukaryotic genomes. Conversely, the variation inherent to specific cancer cell genomes results in context-specific essential genes whose mutation creates cell fitness defects only in a specific genetic background, presumably due to GIs. Importantly, genome-wide screens for GIs offer the potential to convert a given nonessential gene into a context-specific essential gene, and thereby define the genes and pathways that buffer the loss of function of any mutant query gene.

MAPPING GENETIC INTERACTIONS IN CANCER

Generally, a genetic interaction occurs when the fitness phenotype observed for a given double mutant deviates from the phenotype expected based on the two single mutant phenotypes [4]. If the double mutant grows better than expected, the gene pair is said to exhibit a positive GI. If the observed double mutant fitness is less than expected, the two genes display a negative interaction, ranging from synthetic sickness to the most extreme case, synthetic lethality (SL) (Fig. 1A).

Efforts to broadly map GIs in human cells are particularly relevant to understanding cancer phenotypes for two reasons. First, GIs complicate our ability to predict phenotype from genotype, a major challenge that must be addressed to realize the promise of precision medicine for cancer. Second, GIs, and SL in particular, are important as a therapeutic

concept in cancer. The idea of discovering and exploiting specific genetic vulnerabilities to kill cancer cells while sparing normal tissue was proposed more than 20 years ago [5]. Synthetic lethal interactions in cancer cells can be considered a form of context-dependent gene essentiality [2], although it should be noted that the number of modifiers associated with the GI could be complex rather than a simple digenic effect. SL as a therapeutic model has obvious benefits of potentially reducing side effects of cancer treatments, as well as the possibility of indirectly targeting “undruggable” mutations. These ideas have motivated massive academic and corporate cancer SL screening approaches in a vast range of cell systems, yielding thousands of SL interactions [6].

While the promise is huge, only one SL interaction has been translated into the clinical setting to date: as noted above, breast and ovarian cancer cells carrying mutations in *BRCA1* or *BRCA2* are highly sensitive to PARP inhibitors [1]. Even though many promising candidates have been identified in tumor cell models [1,7], most published SL interactions have not withstood pre-clinical evaluation. These failures may result from off-target effects, incomplete loss-of-function and poor reproducibility in RNAi screens [8,9], variable consistency of drug screens [10,11], incomplete penetrance [12], and context dependency [2]. These issues, coupled with the fact that GIs are rare, involving on the order of ~1% of tested gene pairs, suggest that efficient discovery of clinically actionable GIs will require a more global analysis of genetic networks that moves beyond individual genes and pathways.

MAPPING GENETIC INTERACTIONS IN MODEL SYSTEMS: A TEMPLATE FOR GENETIC NETWORK ANALYSIS

Model organisms continue to be experimental test-beds for development and implementation of systematic GI studies due to their small genomes, genetic tractability and amenability to high-throughput analyses. Systematic genetics in the budding yeast *Saccharomyces cerevisiae* enabled assembly of the first comprehensive pairwise GI map surveying nearly all essential and non-essential yeast genes [13]. Most query genes display a number of different negative and positive interactions, and the set of GIs associated with a query gene forms a GI profile. Genes within the same biological pathway or protein complex have highly similar GI profiles, indicating that these profiles provide a quantitative measure of gene function. Indeed, the global map of yeast GI profiles models a powerful approach for annotating gene function, assembling a hierarchical map in which genes are grouped according to functional modules corresponding to pathways and complexes, biological processes, and cellular compartments [13](Fig. 1B). The global yeast GI network can be expanded to include additional layers of complexity, such as genetic suppression [14], trigenic interactions [15], and condition-specific GIs [16] (Fig. 1C). Similar mapping efforts, albeit less comprehensive, have been undertaken in multicellular eukaryotic model organisms, including cells derived from the fruit fly *Drosophila melanogaster*, whole-organism screens in the nematode *Caenorhabditis elegans* and the zebrafish *Danio rerio*.

Coherent sets of negative GIs often occur between genes in two pathways or complexes, which is referred to as a between pathway module (BPM) or within an essential pathway and complex, which is referred to as a within pathway module (WPM) [4], and these network

structures of the yeast GI network motivated the development of a method to infer GIs from human GWAS studies in breast cancer [17]. Moreover, the existence of a synthetic lethal interaction in yeast increases the likelihood of finding an interaction between the human paralogues by 3- to 19-fold [18]. Recently, GIs identified in *Drosophila* cells have been used to guide hypotheses about Wnt signaling in human cancers [19], and a zebrafish GI system helped to establish a role for *SPRED1* in mucosal melanoma [20]. These and other studies affirm the power of insights derived from experiments in model systems for guiding insightful human pre-clinical research.

THE CRISPR REVOLUTION IN MAMMALIAN CANCER GENETIC INTERACTION MAPPING

Since the first reports describing use of the bacterial CRISPR-Cas phage defence system for ectopic genome editing, the number of applications and expansions of the CRISPR ‘tool kit’ has exploded [21], revolutionizing the field of functional genomics and GI mapping (see Table 1). In addition to CRISPR-Cas9-mediated loss-of-function genome editing, systems for introducing point mutations and other targeted modifications, as well as for activating and repressing transcription, have been developed and used to interrogate GIs. Recent “classical” or loss-of-function CRISPR screens have identified new SL cancer targets (Fig. 1D) including: [1] sets of acute myeloid leukaemia-specific essential genes [22]; [2] *ENL* as a specific vulnerability in *MLL-AF4*-positive acute leukaemia [23]; and [3] interactions between *RNF43* and *FZD5* in pancreatic cancer [24]. In addition, a SL interaction of BAF-complex-mutant synovial sarcomas and malignant rhabdoid tumours with a non-canonical SWI/SNF complex has been described [25], and *RB1*-null small cell lung cancer cells are hyper-dependent on aurora kinase [26,27] (see Box 1 for pharmacogenetic interactions). These genetic insights add novel clinically testable hypotheses to the catalogue of cancer SL interactions.

In order to interrogate reciprocal GIs between multiple genes (Fig. 1D), several combinatorial CRISPR-based screening platforms have been developed. For example, dual gRNA systems designed to enable simultaneous knock-out of two genes in the same cell have been used to: [1] interrogate all pairwise interactions of 73 cancer genes in multiple human cancer cell lines to reveal a druggable SL interaction network [28]; [2] survey barcoded dual gRNA combinations targeting 50 genes for their ability to inhibit proliferation of an ovarian cancer cell line [29] and; [3] measure pairwise interactions between ~21,000 druggable genes to generate a large matrix of ~500,000 measurements, identifying potential synergistic drug combinations [30]. Although dual gRNA screens are useful, concerns with respect to introduction of multiple DNA double-strand breaks in conventional Cas screening, as well as relatively poor screening efficiency, have prompted the development of combinatorial screening systems using CRISPR interference (CRISPRi), where endonuclease-deficient Cas9 is used to target transcriptional repressors to gene promoters to simultaneously repress multiple target genes [31]. In one study, CRISPRi was combined with CRISPR activation (CRISPRa), where transcriptional activators are used instead of repressors, by using two different Cas9 proteins from orthologous species to investigate directional GIs in a chronic myeloid leukaemia cell line [32]. A similar system of orthogonal

CRISPR enzymes for activation and repression was used to perform GI screens between apoptosis genes, *MAP* kinases and *AKT* genes in multiple cancer cell lines, identifying a novel SL between *BCL2L1* and *MCL* [33].

CRISPR tools have also been combined with single-cell technology for increased resolution and sensitivity of screens and an expanded repertoire of screenable phenotypes, such as cell lineage tracking using unique molecular identifiers (e.g. [34]). One recent study also used combinatorial CRISPR perturbations in single cells with transcriptomics as a readout [35], providing a useful avenue for larger scale interrogation of GIs in individual cancer cells. Similarly, CRISPR applications have greatly facilitated cancer mouse model generation, and have allowed direct *in vivo* screening for inhibitors of tumour growth, metastasis or interaction with the immune system and microenvironment, as reviewed elsewhere (e.g. [36]). These applications illustrate the power and versatility of CRISPR technology, but have not yet reached the capacity for unbiased, large-scale investigations of GIs.

TOWARDS COMPREHENSIVE GENETIC INTERACTION MAPPING IN HUMAN CELLS

The efficiency and ease of CRISPR technologies have catalyzed major efforts to systematically interrogate genetic dependencies in large panels of cancer cell lines like the Broad Institute's "Cancer Dependency Map Project" (depmap.orgs) that currently contains screens in nearly 500 cancer cell lines. The lessons learned from RNAi applications with respect to quality of reagents, replication of screens, accuracy and robustness of measured phenotypes, as well as data analysis have been invaluable for the rapid development of CRISPR technologies and have led to increased awareness of potential pitfalls that are specific to CRISPR. For instance, multiple groups have reported that targeting amplified genomic regions results in gene-independent reduction of proliferation and viability, suggesting that an increased number of DNA double strand breaks generated by Cas9 causes toxicity [37–39]. However, bioinformatic algorithms can efficiently correct for these and other off-target effects [39,40], enabling integrated analysis of large collections of genome-scale CRISPR loss-of-function screening data. Not only are these datasets a valuable resource for hypothesis-driven data mining, especially with respect to uncovering novel SL interactions, but they also provide the basis for the first attempts towards generating comprehensive co-essentiality networks in human cells. Recent studies have integrated and re-analyzed multiple such datasets to derive cancer GI maps based on CRISPR screen and mutation data [41]. Similar to the analysis of GI profiles from the global yeast GI network, these efforts facilitated the annotation of protein complexes [42] and inspired the proposal that a hierarchical GI network for human cells can be extracted from cancer co-essentiality data [43,44] (Fig. 1D).

While CRISPR has virtually replaced RNAi in functional genomics applications, RNAi technology itself has seen recent technical improvements and computational off-target correction methods for large datasets [45–47]. This has catalyzed further projects to map cancer dependency maps using RNAi within the frameworks of "Project Achilles" and "Project DRIVE" [45,46], whose data are being integrated into the DepMap Project. These

initiatives map genetic perturbations that cause a vulnerability in the form of a fitness defect in certain cancer cell lines, but do not directly measure GIs between defined pairs of genes. Nevertheless, systematic pairwise GI mapping in human cells with defined genetic backgrounds has been reported for a small set of cell lines [48,49] and pairwise GI mapping in hundreds to thousands of defined “single mutant” model cell lines is within reach (Fig. 1D). Recently, a first blueprint of a human GI landscape was produced based on experiments in two cancer cell lines [50]. The authors used CRISPRi to perturb over 200,000 gene pairs and used the resulting GI maps to annotate uncharacterized genes by functional clustering, an approach that mirrors GI mapping in yeast (see above). The project also identified novel cancer-relevant negative GIs, demonstrating that large-scale analysis in human cells is feasible and likely to provide rich functional information, facilitating annotation of the normal human and cancer cell genomes (Fig. 1D). Ultimately, the comparison of multiple CRISPR and RNAi datasets, coupled with experimental and clinical validation of the screening results, will provide the community with a clear view of the robustness of different technologies for a chosen biological question or clinical application.

CONCLUSIONS AND OUTLOOK

CRISPR technologies have not only revolutionized the study of individual genes, but also large-scale investigations of gene essentiality and GIs, resulting in an ever-expanding toolbox of experimental and computational methods to find genetic vulnerabilities in cancer cells. Despite enormous technical progress, major challenges remain: the complexity and size of the human genome hampers the generation of global GI maps, benchmarks for robust and clinically relevant GIs remain to be developed, and the context-dependency of GIs in patients, tissues and cell lines represents an important hurdle for cogent mapping of genetic networks.

However, technology development will continue to drive innovations for human GI mapping, as has been the case for mapping of genetic networks in model systems. For example, the integration of other phenotypic readouts beyond cell proliferation holds great promise. Direct readouts include assays of protein-protein interactions or protein levels and modifications [51,52]. Quantitative data-based experimental pipelines that combine automated image analysis and systematic genetic perturbation have been productively used to explore bioprocess-specific GIs in yeast and fly [53,54] and comparable methods that involve optical barcoding and *in situ* sequencing have been recently developed for mammalian cells, enabling phenotypic profiling at single-cell resolution [55,56]. Single-cell technology will mature and allow more complex phenotyping, for instance by barcoding on the protein level and mass cytometry analyses [57]. A logical expansion of “deep-mutational-scanning”-like technologies, where individual genes are mutagenized to saturation, to the interrogation of allele-specific GIs should prove highly informative. Finally, the growing CRISPR toolbox will be exploited for engineering more precise genetic lesions for use in GI analyses by base editing or by using engineered or orthologous Cas enzymes with altered or expanded target site preferences [21]. These technologies will allow for conditional GI screens, similar to model systems [16], time resolved or gene dosage screens [54,58], and multiplexing for interrogation of higher-order GIs [59]. Importantly,

most of the described applications are in principle also applicable to *in vivo* or 3D-culture systems such as organoids, albeit at smaller scale.

The application of diverse methods for genetic network analysis in cancer will require advanced strategies for data integration, which ultimately will result in improved coverage of gene (protein) interactions, as suggested by maps generated by combining genetic with protein-protein interaction and phosphoproteomics data [60,61]. The ever-growing repository of cancer genomics and GWAS data has sparked the development of approaches for data compilation and data mining or candidate prioritization [62], which will soon be enabled by powerful deep learning technologies to model cellular networks [63]. A global understanding of human genetic networks in healthy and diseased cells and tissues demands a systematic community effort, and will lead to new mechanistic insight into cancer, generate informed, clinically testable hypotheses and, ultimately better therapies.

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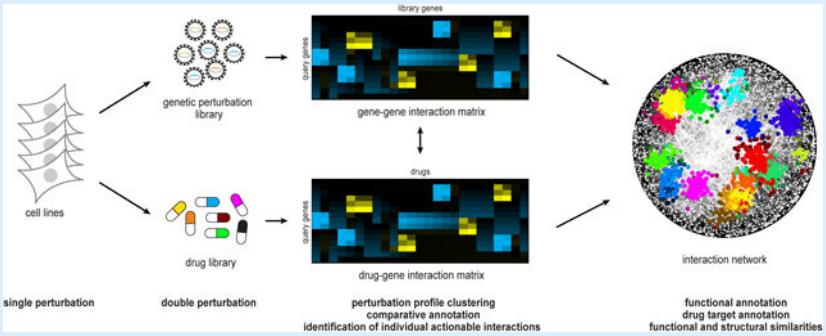
Box 1: Pharmacogenetic interactions

In addition to mapping gene-gene interactions, CRISPR genome editing has been used to dissect gene-drug or pharmacogenetic interactions. Pharmacogenetic interactions can mimic genetic interactions, but genetic interactions and networks can also be used as a template for interpreting drug-gene interactions, facilitating target identification (diagrammed below). Challenges include altered phenotypes relative to genetic perturbation due to drug off-target effects and inherent differences between pharmacologic inhibition and genetic ablation of a gene/protein of interest. Nonetheless, the identification or confirmation of a genetic interaction using a drug facilitates validation of a pharmacological target for pre-clinical investigations. If the drug displaying the desirable interaction is already in clinical development or approved, the subsequent development process will be accelerated considerably. Recently published pharmacogenetic screens include classic synthetic lethality screens [27] but also efforts using dual-gRNA systems to investigate potential drug synergies or resistance mechanisms [30,32]. On a larger scale, pharmacogenetic screens in hundreds of cancer cell lines are being performed within the framework of ongoing consortium projects listed below. These initiatives aim to produce a comprehensive view of pharmacogenetic interactions in cancer and may be expanded to organoid or *in vivo* systems.

Pharmacogenomic Screening Consortia:

- Genomics of Drug Sensitivity in Cancer (GDSC) –www.cancerrxgene.org
- The Connectivity Map (CMAP) –www.broadinstitute.org/connectivity-map-cmap
- The Genentech Cell Line Screening Initiative (gCSI) – [11]
- The Cancer Therapeutics Response Portal (CTRP) – portals.broadinstitute.org/ctrp
- The Cancer Cell Line Encyclopedia (CCLE) - portals.broadinstitute.org/ccle
- The Cancer-Drug eXplorer (cDx) - cancerdrugexplorer.org



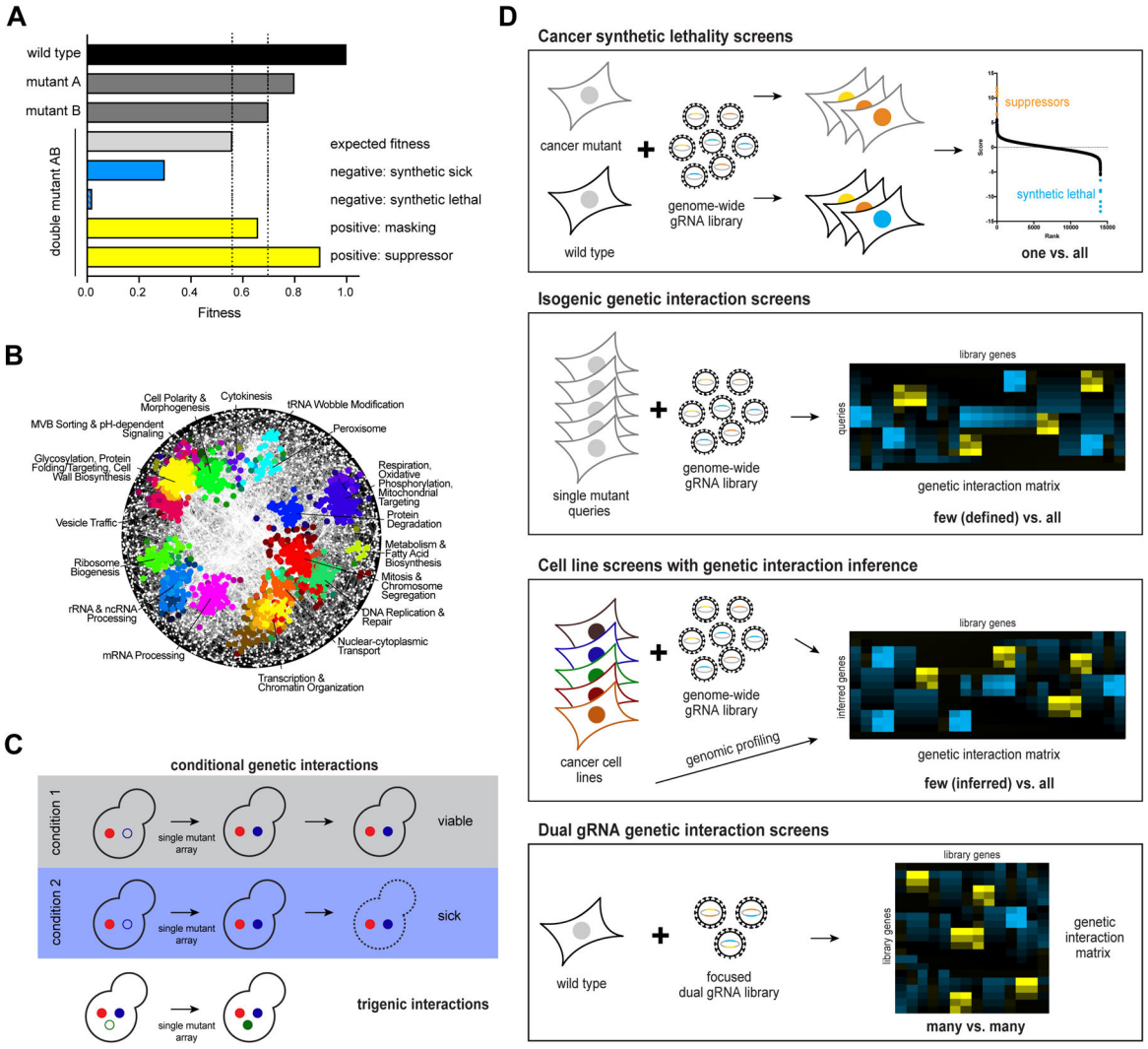


Figure 1: Genetic interaction mapping.

(A) Schematic illustration of genetic interactions as measured by single mutant and double mutant fitness. Negative genetic interactions result in lower double mutant fitness than expected (synthetic sick, synthetic lethal), positive genetic interactions in greater fitness than expected (masking or suppressive). **(B)** Global pairwise genetic interaction network in yeast reveals functional clustering of genes with similar genetic interaction profiles and allows annotation of uncharacterized genes. **(C)** Expansion of the yeast functional genomics landscape by conditional and trigenic interactions. **(D)** CRISPR-mediated genetic interaction screens in mammalian cells. Top, gRNA representation in a cell line harbouring a cancer mutation is compared to a wild type cell line to identify synthetic lethal or suppressive interactions. Second, a limited number of defined mutants are generated in an isogenic cell line background and subjected to genome-scale CRISPR screening. Clustering by genetic interaction profile similarity reveals functional information. Third, instead of isogenic mutants, patient-derived cancer cell lines are used. In addition to CRISPR screening, genomic profiling is required to infer the “single mutant state” in those cell lines. Bottom,

Direct assessment of pairwise genetic interactions between a limited set of genes by simultaneous delivery of two gRNAs into the same cell.

Table 1:
Technologies for genetic interaction mapping in mammalian cells.

Overview of current and emerging technologies with potential to aid in genetic interaction mapping in mammalian cells. Corresponding references can be found in main text.

Technology	Description	Application	Type	Variations	References (mentioned in main text)
CRISPR mutagenesis (CRISPRm) or cutting (CRISPRc)	genome editing through Cas enzyme-induced double-strand breaks and endogenous repair; targeting by gRNA	pooled and arrayed loss-of-function experiments; genome-scale screens; single-well mechanistic experiments	genomic (coding sequence)	compatible with multi-targeting approaches; homologous-recombination-mediated knock-in; various natural and engineered Cas enzymes with different properties	
CRISPR interference (CRISPRi)	transcriptional repression by Cas fusion proteins for targeting and repression	pooled and arrayed loss-of-function experiments; genome-scale screens; single-well mechanistic experiments	transcriptional (promoter region)	compatible with multi-targeting approaches	
CRISPR activation (CRISPRa)	transcriptional activation by Cas fusion proteins for targeting and repression	pooled and arrayed gain-of-function experiments; genome-scale screens; single-well mechanistic experiments	transcriptional (promoter region)	compatible with multi-targeting approaches	
CRISPR base editing	mutagenesis of individual bases through modification (e.g. deamination) by Cas fusion proteins	arrayed and single-well experiments; forward-genetic screens	genomic (coding sequence)	multiple Cas and deaminase versions for editing different bases; CRISPR-STOP for engineering stop codons	
epigenetic editing	alteration of chromatin states by Cas fusion proteins with chromatin modifying enzymes	arrayed (possibly also pooled) experiments; single-well mechanistic experiments	epigenomic, transcriptional	multiple modifiers and combinations possible	
combinatorial CRISPR	induction of multiple perturbations in the same cell by multiple gRNAs	pooled and arrayed genetic interaction experiments; genome-scale screens; single-well mechanistic experiments	genomic or transcriptional	combinations of CRISPR, CRISPRi and CRISPRa; orthologous promoters or Cas enzymes	
Perturb-Seq, CROP-Seq	CRISPR-mediated induction of perturbation coupled to single-cell RNA sequencing	pooled loss- or gain-of-function experiments	genomic (single cell)	different methodologies; compatible with different CRISPR systems	
mutational scanning	high-density CRISPR-based mutagenesis	pooled loss- or gain-of-function experiments for single genes	genomic (coding sequence)	different mutagenesis methods possible	
unique molecular identifiers	single-cell barcoding (combined with CRISPR-mediated perturbations)	pooled and arrayed loss- or gain-of-function experiments; genome-scale screens; single-well mechanistic experiments; lineage tracking	genomic (single cell)	compatible with various CRISPR systems	
optical barcoding	imaging-based readouts coupled to barcoding and *in situ* sequencing	pooled and arrayed phenotypic experiments; genome-scale screens; single-well mechanistic experiments	genomic (single cell)	multiple phenotypes and various technical options conceivable	

Technology	Description	Application	Type	Variations	References (mentioned in main text)
protein barcoding (e.g. CITE-seq)	integration of protein-tags as barcodes, measured by antibody-mediated CyTOF technology	pooled and arrayed phenotypic experiments; genome-scale screens; single-well mechanistic experiments	genomic/ proteomic (single cell)	multiple phenotypes possible	