Proteome-wide chemical-genetic interaction map in human cells reveals drug mechanisms and novel gene functions

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Chemical-genetic interaction screens in yeast have uncovered informative phenotypes for the majority of genes and helped characterize drug mechanisms of action (MOA). The repurposed CRISPR-Cas9 system has recently unlocked the potential to perform similar screens in human cells. We have built a high-complexity gene knockout sgRNA library targeting 19,084 human genes, 3,872 uncharacterized predicted ORFs and 20,852 alternatively-spliced exons, which we introduced into a Cas9-inducible B-cell lymphoma clonal cell line. In order to systematically identify functionally informative chemical-genetic interactions, we screened this library under 144 different chemical treatments (Figure 1), including 102 compounds with varied mechanisms of action (MOA) covering a broad range of cellular functions.

In addition to chemical-genetic interactions with non-essential genes, our system can also identify chemical-genetic interactions involving essential genes, which are central to many drug MOAs, cancer progression and cell biology. We show that this requires controlling for the growth-dependent dropout of essential gene-targeting sgRNAs. We developed a novel approach which leverages the number and diversity of different screens performed to control for this effect as well as for other sources of noise.

We thus identified thousands of high-confidence chemical-gene interactions, including for thousands of splice-variants and hundreds of previously uncharacterized genes. Hypothetical protein-coding genes with strong chemical-genetic interactions were significantly more likely to show evidence of protein expression, demonstrating the potential of our approach for identifying new genes. We show that pairs of genes with similar chemical-genetic profiles tend to share functions or to encode physically interacting proteins. This correlation allows us to predict potential functions and interactions for thousands of genes, including many with little or no functional annotations, as well as to decouple the functions of alternatively-spliced isoforms.

We also found that screening the same compound at different doses can reveal complementary information, and sometimes produces dramatically different signatures. We demonstrate the existence of a generic chemical-induced stress response, whereby particular genes tend to interact with a wide array of different compounds. For compounds with known MOA, we found that screens tend to cluster together by MOA, demonstrating the potential to characterize unknown MOAs. For many screens, the list of genes showing the strongest synergistic or antagonistic interactions clearly captures the pathway known to be involved in drug MOA. Notable examples include apoptotic responses, microtubule polymerization, the spindle checkpoint, cullin neddylation, nucleotide metabolism, translation, the mitochondrial respiratory chain, the ubiquitin-

proteasome system and DNA damage/repair. Our results allow us to assign many additional protein functions to these pathways, thereby greatly extending currently-available annotations.

We observed that chemical-genetic interactions are rarely conserved from yeast to human and that chemical-genetic profile similarity between pairs of genes is also significantly but weakly conserved from yeast to human. This result indicates that cellular networks have substantially rewired throughout evolution, such that drugs can often exhibit highly distinct MOAs in distant species, and highlights the importance of performing these screens in human cells.

This study establishes that chemical-genetic screening of pooled CRISPR-Cas9 gene knockout libraries can effectively be scaled up both in terms of library complexity and number of compounds to systematically characterize unknown drug MOA, identify novel gene functions, deconvolve splice-variant functions and discover new genes. The chemogenomics strategy demonstrates the power of unbiased genome-wide approaches to characterize protein function and biological responses.

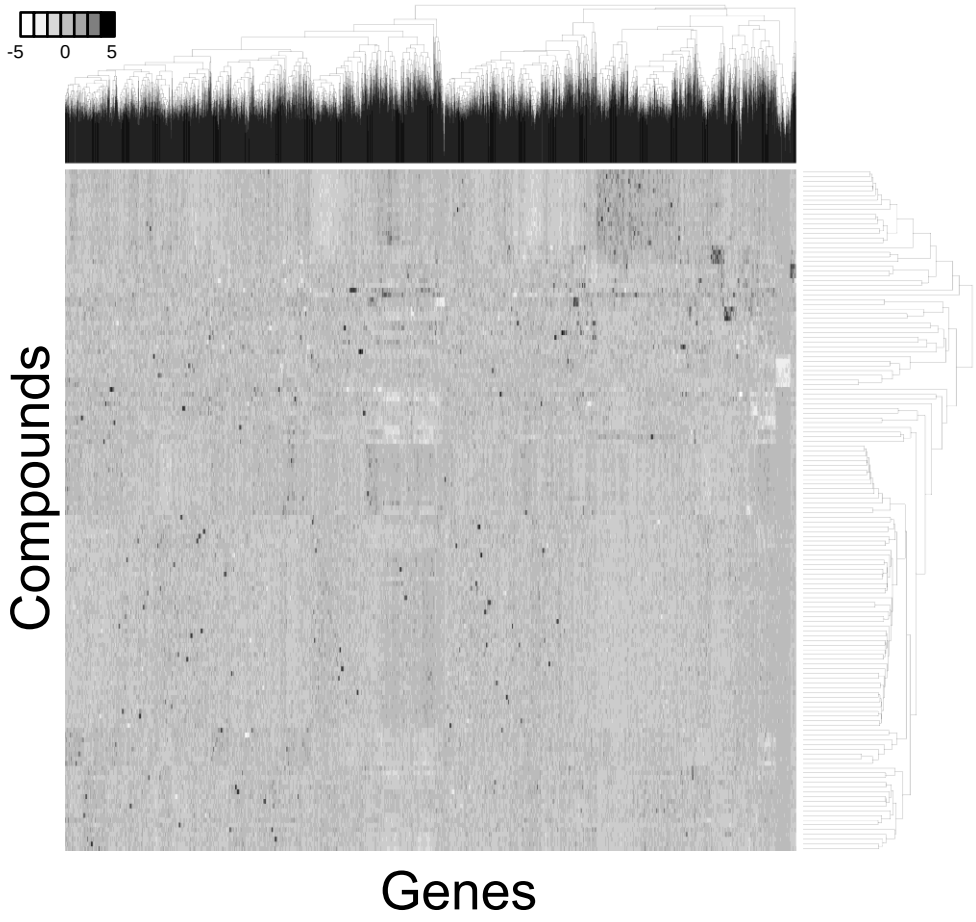

Figure 1: Heatmap displaying sgRNA depletion (white) or enrichment (black) scores for 6,032 genes with chemical-genetic interactions across 144 chemical treatments.