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Chemical genetics in drug discovery

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Abstract

Chemical-genetic approaches are based on measuring the cellular outcome of combining genetic and chemical perturbations in large-numbers in tandem. In these approaches the contribution of every gene to the fitness of an organism is measured upon exposure to different chemicals. Current technological advances enable the application of chemical genetics to almost any organism and at an unprecedented throughput. Here we review the underlying concepts behind chemical genetics, present its different vignettes and illustrate how such approaches can propel drug discovery.

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High-throughput screening, Drug target, Drug interactions, Genomics, Drug resistance.

Introduction

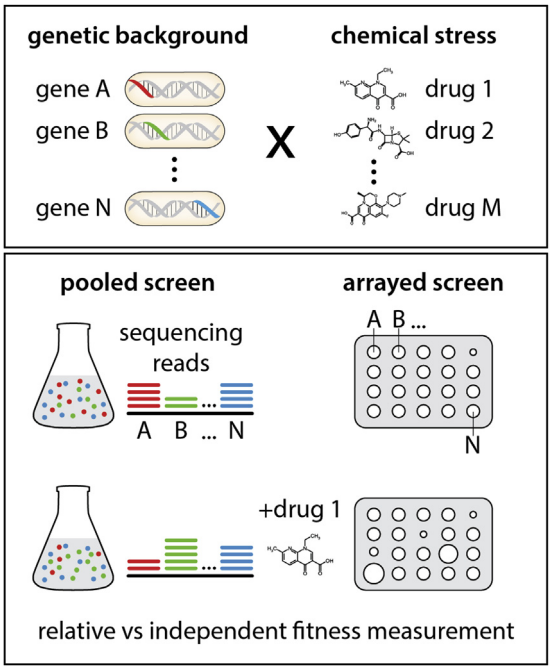
Chemical genomics and *chemical genetics* are often used interchangeably in literature. *Chemical genomics* is a broader umbrella term describing different types of large-scale *in vivo* approaches used in drug discovery, including *chemical genetics* but also large-scale screening of compound libraries for bioactivity against a specific cellular target/phenotype. In contrast, the term *chemical genetics* refers specifically to the systematic assessment of the impact of genetic variance on the activity of a drug (Figure 1). Chemical genetics was pioneered in microbes [1–4], but is now increasingly applied in human cell lines [5,6]. The focus of this review will remain on chemical-genetic approaches in microbes, briefly introducing the enabling tools and highlighting the benefits of these applications to drug discovery – identification of Mode-of-Action (MoA), mapping of uptake and efflux routes, revealing of resistance mechanisms and understanding of interactions with other drugs.

Basis of chemical genetics

High-throughput reverse genetics approaches, such as chemical genetics, have been propelled by the revolution in our ability to generate and track genetic variation for large population numbers. Genetic variation used in such screens comes in many flavors, ranging from controlled to natural. In its most powerful iteration, genome-wide libraries containing mutants of each gene in the chromosome are profiled for changes in the effect of a drug to the organism. Such libraries consist of loss-of-function (LOF; knockout, knockdown) or gain-of-function (GOF; overexpression) mutations and can be arrayed or pooled (Figure 1). In the past decade mutant libraries have been constructed in a plethora of bacteria and fungi [7]. More recently, our proficiency in generating genome-wide pooled mutant libraries [8] and deconvoluting via multiplexing sequencing approaches [9,10] has brought us to a stage where libraries can be created for almost any microorganism [11]. Although natural genetic variation is frequently used in chemical genetics in human cell lines [5,6,12], this unlimited resource has only been recently explored in bacteria [13], leading to similar abilities to delineate drug function as ordered libraries.

To perform reverse genetics in large-scale, creating systematic genetic variance is not enough; one needs to also quantitatively phenotype these populations. Barcoding approaches, pioneered in bacteria [14] and perfected in yeast [15], together with advances in sequencing technologies, have allowed for tracking the relative abundance, and thus the fitness of individual mutants in pooled libraries with unprecedented throughput and dynamic ranges [16,17]. Thereby differences in relative abundances of mutants in the presence and absence of a drug can reveal genes required or being detrimental for the organism to withstand the drug's cytotoxic effects [1,18]. Similarly, experimental automation and image processing software [19,20] allows for chemical genetics in arrayed libraries, where hundreds to thousands of mutants are profiled on the same plate [2,4,21]. In arrayed formats, the effects of drugs can be assessed by additional macroscopic phenotypes other than growth, including developmental processes, such as biofilm formation and sporulation, DNA uptake, or cell lysis [20,22,23]. Although microbial chemical-genetic screens have concentrated on measuring bulk phenotypes, quantifying single-cell phenotypes and population behaviors across mutant

Figure 1



Basic concepts and approaches in chemical genetics. Chemical-genetic approaches are based on the combination of genetic and chemical perturbations. The fitness of genome-wide libraries of gain-of-function and loss-of-function mutations is assessed upon exposure to large numbers of drugs. Mutant libraries can be pooled or arrayed. In pooled screens barcoded mutants compete among each other after exposure to a certain drug, and their relative abundance is measured by barcode sequencing. In arrayed screens mutants are ordered and their fitness or additional macroscopic phenotypes can be assessed in an independent fashion.

libraries is also possible with current advances in high-throughput microscopy [24,25]. In such cases, cell markers and classifiers of drug responses can provide further insights into the biological activity of the drug in the cell [26]. Single-cell readouts and multi-parametric phenotyping analysis are more common in chemical genetics in human cell lines [12].

Chemical genetics in MoA identification

There are two main ways that chemical genetics can be used to map drug targets. First by using libraries in which the levels of essential genes, the usual target of drugs, can be modulated. In this case, when the target gene(s) is down-regulated the cell often becomes more sensitive to the drug (as less drug is required for titrating the cellular target), and the opposite holds true for target gene overexpression (Figure 2). For diploid organisms, heterozygous deletion mutant libraries can be used to reduce the dose of essential genes. Such screens, dubbed as HaploInsufficiency Profiling (HIP), were the first to be used to successfully map drug cellular targets in yeast [3,27,28]. As bacteria are haploids, increasing gene levels is technically simpler. Thus, target overexpression has been repeatedly used to identify the target of new drugs [28–30]. Recently, with the advance of CRISPR-based technologies, CRISPRi libraries of essential genes have been constructed in different bacteria [31,32] and used for identifying drug targets [31]. Compared to overexpression approaches, knockdown libraries of essential genes have the advantage of being better tailored for capturing the cellular target when this is part of a protein complex (Figure 2). Nevertheless, both approaches have caveats, as genes conferring directly/indirectly resistance to the drug are also detected as hits, and steady-state experiments after induction or down-regulation of an essential gene may result in more drastic effects in the cellular network than just changes in the levels of the gene targeted. Such caveats can be partially overcome by combining results of increased and decreased gene dosage [33] and by more generally titrating gene dosage, or by checking dynamic responses after modulating the levels of essential genes. Nevertheless, more complex drug-target relationships may remain unresolved by simply changing the target levels [34]. Knockdown and overexpression approaches are now starting to be used to identify drug targets in human cells lines [35,36].

A second way to infer the drug target from chemical genetics data is by comparing drug signatures [2,4]. A drug signature comprises the compiled quantitative fitness scores for each mutant within a genome-wide deletion library (all non-essential genes) in the presence of the drug. Drugs with similar signatures are likely to share cellular targets and/or cytotoxicity mechanisms [2,4,21]. This guilt-by-association approach becomes more powerful when more drugs are profiled, as repetitive "chemogenomic" signatures, reflective of the general drug MoA, can be identified [18]. Yet, drug signatures are driven by pathways controlling the intracellular drug concentration as much as they depend on pathways related to drug MoA or its cytotoxic effects to the cell. Thus, machine-learning algorithms can be used to recognize the chemical-genetic interactions that are reflective of the drug's MoA. Although not yet used in such applications, Naïve Bayesian and Random Forest algorithms have been recently trained with chemical genetics data to predict drug–drug interactions [37,38]. Finally, although single-cell morphological profiling can be very powerful for MoA identification on its own [26,39], it has not been used yet as a readout for large-scale chemical genetic screens in microbes. Small-scale screens do exist [40] and morphological profiling of wildtype cells has been combined only to a limited degree with growth-based chemical genetics [41]. In contrast, multi-parametric analysis of microscopy images is common for chemical genetic screens in cell lines, increasing the resolution for MoA identification [12]. Moving similar concepts to microbial chemical genetics is bound to improve our capacity for MoA identification.