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Phenotypic screening with primary neurons to identify drug targets for regeneration and degeneration

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ABSTRACT

High-throughput, target-based screening techniques have been utilized extensively for drug discovery in the past several decades. However, the need for more predictive *in vitro* models of *in vivo* disease states has generated a shift in strategy towards phenotype-based screens. Phenotype based screens are particularly valuable in studying complex conditions such as CNS injury and degenerative disease, as many factors can contribute to a specific cellular response. In this review, we will discuss different screening frameworks and their relative utility in examining mechanisms of neurodegeneration and axon regrowth, particularly in cell-based *in vitro* disease models.
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1. Introduction

Although drug screens based on cellular, organ, or whole-animal phenotypes once dominated the drug discovery landscape, the revolution in molecular biology and genomics resulted in this approach being supplanted by screens targeting defined proteins implicated in disease (Kotz, 2012). Over the past 15 years, the most common drug discovery approach has been the target-based screen, in which large

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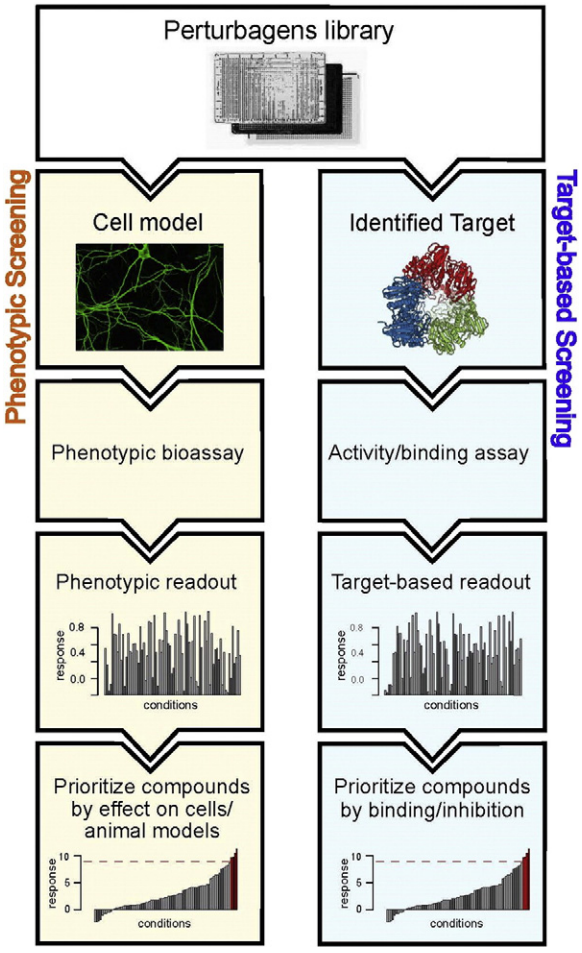

Fig. 1. Target-based *versus* phenotypic screening approaches. Two major approaches dominate drug discovery. The first, target-based screening, seeks novel compounds to alter that activity of a validated target. In this approach, the compounds are typically tested in a biochemical assay against a single target and the compounds are prioritized based on the readouts of the assay. The biggest limitation in this process is that the targets must be identified and validated before a screen can be considered. In a phenotypic screen, the perturbagens are screened in cellular models and they are prioritized based on phenotypic readouts. It doesn't require a target-based hypothesis, allowing discovery of highly effective perturbagens in an agnostic approach. However, targets may be difficult to identify and sometimes they remain unknown. If the perturbagens are compounds, an effort is then made to determine their molecular targets.

numbers of compounds are screened for binding to or altering the activity of a single target protein. Subsequently, hit compounds are optimized through medicinal chemistry efforts to define structure-activity relationships (SAR) as well as to improve pharmacokinetic parameters (Khurana et al., 2015; Drews, 2000; Lee and Bogyo, 2013). Despite this dominance, phenotypic (cell-based) screening has recently made a comeback, as an approach to discover biologically active hits relevant to a biological process or therapeutic outcome (Lee and Bogyo, 2013). In particular, phenotypic screening of neural cells offers a way to find compounds or gene targets that modulate the key phenotypes of neurodegeneration and neuroregeneration, without the requirement for the detailed mechanistic knowledge that is often lacking in complex neurological disorders (Zhang et al., 2014; Swinney and Anthony, 2011; Khurana et al., 2015; Rosamond and Allsop, 2000). Multiple neurodegenerative diseases have been studied both with target-based and phenotypic-based screens, including Alzheimer's Disease (AD; Bettens et al., 2010), Parkinson's Disease (PD; Cookson and Bandmann, 2010), bipolar disease, autism, and schizophrenia (Haggarty et al., 2016).

2. Review

2.1. Target-based versus phenotypic drug screening

While both target-based and phenotypic-based screens can be utilized for drug discovery, each methodology has distinct advantages and disadvantages (Fig. 1). For compound screening, cell free, target-based assays are typically simpler to execute than phenotypic assays, provide quantitative results based on simple reporter systems, and clearly implicate a specific molecular target. Ideally, high-affinity compounds are identified with a known target and mechanism of action, but such targets must obviously be identified and validated before a screen can be developed; novel targets cannot be identified (Swinney and Anthony, 2011; Khurana et al., 2015). Well-designed phenotypic assays, in contrast, identify hits that are related to a biological process or disease state, and can also identify compounds that have undesirable features or effects, such as toxicity, poor cellular permeation and, of course, lack of biological (as opposed to biochemical) efficacy. An advantage of phenotypic screening compared to target-based screens is suggested by the relative numbers of drugs identified with novel molecular mechanisms of action (MMOA) that have been approved by the Food & Drug Administration (FDA) (Frantz, 2005). An important limitation of the phenotypic approach is that the cellular models require a robust and consistent phenotype that is clearly correlated with the pathology for which therapy is sought, and is measurable by automated microscopy (Swinney and Anthony, 2011; Khurana et al., 2015).

Once a therapeutic target has been identified, it needs to be validated, both *in vitro* and in animal models (Hughes et al., 2011; Tardiff and Lindquist, 2013). Target-based biochemical screening depends on knowing the exact target and the MMOA associated with a well-defined pathology (Sams-Dodd, 2005), and hypothesizes that varying the target activity will also modify the disease (Tardiff and Lindquist, 2013; Khurana et al., 2015). The process of target validation (Blake, 2007) could be done using structure-activity relationships (SAR) of analogs of a lead compound, generating a drug-resistant mutant of the presumed target, or knockdown or overexpression of the presumed target and monitoring the known signaling systems downstream of this target.

Evaluating SAR of small molecules is a key task in medicinal chemistry, following identification of a small molecule as a hit. Previously, SAR investigations were focused on individual compounds, but recently scientists interested in characterizing SAR have begun to investigate SAR by bioinformatic analysis, combining results from a large number of compound data sets and different target-based screens (Hughes et al., 2011; Stumpfe and Bajorath, 2012; Keiser et al., 2009). In the future, this analysis will probably be enhanced by combining data from target-based and phenotypic screening (Stumpfe and Bajorath, 2012).

In the past 10 years, the scope of *in vitro* phenotypic screening has been expanded by the advent of "disease-in-a-dish" modeling offered by induced pluripotent stem cells (iPS; Takahashi and Yamanaka, 2006). iPS cells offer a source of disease-specific cells that can be differentiated and examined for molecular mechanisms, physiology, and screening (Unternaehrer and Daley, 2011) of the disease phenotype. This is particularly advantageous for neurodegenerative diseases, as it is often difficult to obtain primary, affected cells from patients, especially in high enough numbers to allow for screening procedures (Unternaehrer and Daley, 2011). Additionally, the use of iPS cells from human patients provides more direct clinical relevance for any screened targets that facilitate phenotypic changes. Indeed, the availability of human iPS cells has redefined the threshold for interest from the biotechnology industry in potential therapies relating to nervous system disorders. Results on a new compound or genetic manipulation, no matter how promising, must be demonstrated on human neurons to be worthy of serious interest.

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