

Resurgence of Phenotypic Screening for Discovering Drug Leads

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Received: March 17, 2017; **Published:** April 05, 2017

Historically, drug discovery relied on phenotypic drug discovery (PDD), also known as “classical pharmacology”, which represented a practical approach where compounds were screened on intact cells or organisms to identify hits that resulted in the desired therapeutic effect. The ease of application, simplicity and rapid findings of such methods resulted in phenotypic screenings becoming the methods of choice for drug discovery for decades.

However, with the arrival of gene cloning and the sophistication of biological techniques in the 1980s, it quickly became possible to adopt more rational, hypothesis-based screening strategies that directly targeted distinct disease-related proteins. This approach, known as target-based drug discovery (TBDD) allowed scientists to step out of the “black box” approach of PDD and avoid the consequence of identifying hits with elusive mechanisms of action. One poignant example of this elusive issue from PDD is the case of acetylsalicylic acid (aspirin) for which the mechanism and molecular target remained unclear for almost a century [1]. Therefore, the appeal of TBDD approaches combined with their potential for higher-throughput screens caught the interest of the pharma industry and became the predominant lead generation strategy for years [2], leaving PDD as a lower priority approach.

TBDD involves the screening of a large library of compounds against a target protein, followed by the optimization of hits to improve potency, selectivity, cellular activity and pharmacokinetic properties [3]. However, in order to be effective, such an approach relies heavily on the assumption that a disease pathology is well-defined and that the target protein is highly validated and essential. In fact, the success of TBDD depends on the correct assumption and validation that link the target with the disease, which has often been fruitful, but sometimes not. The latter is not surprising given that whole living systems are highly complex and often display important potential of adaptations, especially in cases of multifaceted diseases [4]. Furthermore, some diseases may have limited numbers of validated target proteins, such as the case for parasitic infections. Another serious limitation is the great time and efforts that must be expended to render a lead inhibitor of a protein to a viable drug for treating a disease. Beyond protein inhibition, many iterations of compound optimization and testing are required to overcome issues of specificity, cellular penetration, availability, metabolism and efflux membrane transporters, to name only a few of many [3].

A significant advantage of TBDD is the design paradigm of “one drug, one target” which attempts to maximize specificity toward the target protein and reduce off-target side-effects. This strategy has certainly been successful, however, alternate chemical properties such as compound aggregation [5] and others has led to unexpected promiscuity and toxicity. For example, a study of drug-protein interactions involving 890 FDA-approved drugs found that 788 shared molecular targets with at least one other drug. Moreover, extensive mining of seven databases indicated that each drug interacts with an average of six known molecular targets [2,6]. Therefore, the ideal of designing highly specific “lock-and-key” drugs is increasingly being questioned.

With the advances in the “omics era” and instrumental technologies, PDD has recently regained popularity, partly because of the enablement of increasingly higher-throughput PDD assays. Modern phenotypic screens rely mainly on three features: cell viability assays (e.g. microbicidal), signaling pathway assays (partially phenotypic) (e.g. MAPK) or disease-related phenotypic assays (e.g. neurite outgrowth assay for neurodegenerative diseases).

One major advantage of PDD is that positive drug hits identified by cell-based assays inherently satisfy many critical requirements necessary for targeting cell-invasive diseases - such as cell-membrane penetration, stability in biological environment, and toxicity [7]. In fact, hits from cell-based assays can be fast-tracked for optimization given that they readily get into cells, without being metabolized too quickly or being openly cytotoxic at effective concentrations [8]. The optimization cycle involving medicinal chemistry and activity testing can be rapid and thus allow for establishing effective structure-activity-relationships (SAR) that is essential for the drive toward the clinic.

A recent historical perspective is interesting to compare the impact of PDD versus TBDD. A review of the pharmaceutical sector showed that out of the 176 small molecule, new molecular entities registered with the FDA between 1999 and 2008, most of these were discovered using TBDD. Nonetheless, a significant proportion were discovered through PDD and in the case of First in Class molecules, 37% were discovered using phenotype screens versus 23% for target-based screens [9]. Moreover, since significantly more efforts were deployed for TBDD during this period [10], it is likely that the success rate of PDD could be underestimated.

PDD also has the advantage of not being biased toward a small number of potential targets and even has the potential to affect a combination of targets at the same time. As a result, PDD could stimulate research in more “outside the box” areas and even potentially generate new molecular targets while at the same time thinning the herd of companies chasing the same targets. More specifically, phenotypic screening is likely to represent a promising method in order to advance drug discovery for many rare diseases which tend to be under studied, as well as in the context of complex neurological diseases such as Alzheimer’s and Parkinson’s diseases for which there have been many failures of TBDD candidates in clinical trials [11].

One major obstacle in PDD approach is in identifying the underlying mechanisms of action and the specific target protein. Regardless of new tools available, identifying the exact molecular mechanism of action of a compound is still a difficult task, even though this process can be easier in the case of infectious diseases in which resistant mutants can be identified, although even this may not allow for complete determination of the mechanism. Yet, identification of molecular targets is not necessary for regulatory agencies around the world to approve a new drug, as long as it is efficacious and safe for patients [11,12]. One should therefore keep an open mind and not necessarily discard potential new avenues for treatment just because we don’t fully comprehend a compound’s mode of action.

Hence, PDD can potentially identify new drug targets which can be complementary to TBDD as reviews of FDA approved drugs revealed that there are currently ~500 effective drug targets even though the Human Genome Project has revealed a total of ~20,000 human genes encoding close to 500,000 proteins [11]. There is therefore a great deal of potential for new targets discovery and we have possibly just uncovered the tip of the iceberg.

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Volume 7 Issue 3 April 2017

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