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Phenotypic Assays in Drug Discovery: A Resurgence

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Abbreviations: PDD: Phenotypic Drug Discovery; HTS: High Throughput Screening; GPCRs: G-protein coupled receptors; TDD: Target based Drug Discovery; NCATS : National Center for Advancing Translational Sciences; NIH: National Institutes of Health; DARPA: Defense Advanced Research Project Agency ; FDA: Food and Drug Administration; iPSCs: Induced Pluripotent cells

Introduction

Drug discovery efforts in the pharmaceutical industry prior to the 1980's relied on the use of whole animals or organ explants. Typically, compounds or classes of compounds synthesized by chemists specifically for Pharmacy applications or for another industry such as the dye industry were tested in an array of assays that relied on phenotypic changes (morphological, physiological, electrophysiological, etc.) observed in animals or organ explants or tissue extracts [1]. This approach has led to a set of powerful drugs that are in use even today [2]. These leading discoveries have also served as the starting points for other drugs in the class based on chemical modifications of the original discoveries. Thus, in this era, drugs were discovered entirely using Phenotypic Drug Discovery (PDD) approaches.

However, since the advent of recombinant DNA technology in the 1980's, the approach to drug discovery acquired a new facet [3,4]. Advances in molecular and cell biology have contributed to understanding of major biological pathways, biochemical/physiological/genetic mechanisms of disease causation and identification of "drug gable" targets. Ever since, a substantial proportion of small molecule drug discovery has relied predominantly on high throughput screening (HTS) against specific recombinant target proteins such as purified enzymes (e.g., kinases), and G-protein-coupled receptors (GPCRs) and ion channels, expressed in artificial recombinant cells systems, resulting in many novel drugs in every sector of medicine. This approach, in contrast to PDD that it superseded, has been dubbed Target-based Drug Discovery (TDD) [4,5].

Both TDD and PDD have their respective strengths and weaknesses [4]. TDD is a reductionist approach that relies on modulating a specific target protein aided by complete structural and functional information and a mechanistic understanding of its role in a biochemical pathway and in disease, often deciphered using gene knock-out and/or gene over expression in transgenic animals. This knowledge facilitates the creation of efficient engineered screening systems that can be interrogated using high throughput methods permitting the screening of large numbers of compounds. Availability of structural information about the target enables further refinement of hits discovered by TDD through molecular biological, biochemical, structural and in silico approaches; hence TDD generates the sense of a 'rational' approach that is alluring to scientists that contrasts with the 'blind' screening engendered by PDD. TDD's weakness lies in its rather narrow focus on to single target in an engineered system removed from cellular situation relevant to the complex disease state. Because of the complexity of biological networks and the incomplete understanding of the networks, TDD can result in the failure of the putative drug in treating the disease despite its high affinity for its target. In fact, an examination of the approved drugs from the earlier era discovered through PDD approaches shows that many of them show 'poly pharmacology', i.e., interaction with multiple targets in achieving their efficacy as well as in causing side effects[6];it is estimated that on average, current drugs interact with six different targets[7]. Thus, it is clear that despite their discovery based on a single target, drugs produced by TDD interact to various degrees with family members of the primary target as well as with unknown targets [7,8].

Conversely, PDD depends on phenotypic changes as its readout without detailed knowledge of the disease mechanism or target, and hence is target agnostic. Therefore, screening in the PDD mode interrogates the system as a whole and thus has the potential of uncovering compounds that may act on multiple targets relevant for the disease than on a single target in TDD. This can result in the identification of compounds that act via different mechanisms or through poly pharmacology. However, a lack of knowledge of the target(s) and the molecular mechanism of action of the compounds

makes refinement of the hits harder than in the TDD approach. Further, PDDs have significantly lower throughput relative to TDD. However, even when TDD remained the focus, many of the assays of PDD were/are used for secondary validation of TDD hits [4-9].

The advent of TDD following the recombinant DNA revolution and the high expectation placed on TDD overshadowed the use of PDD in the Pharma industry. Many authors have attributed the lack of productivity in the Pharma industry to the higher emphasis on TDD at the expense of PDD [4]. Nonetheless, an analysis of new molecular entities among the drugs approved by the FDA between 1999 and 2008 shows that among a total of 45 first-in-class drugs, a high proportion have been discovered using PDD (37% vs 23% via TDD)[4]. Although the exact numbers on the relative productivity of PDD vs. TDD are debated, it remains that despite the allure of TDD, PDD has continued to be vital part of drug discovery and has drawn increasing attention over the years [7].

In addition to the increase in the use PDD that is apparent in the Pharma industry, PDD's prospects are brighter because of further advances in molecular and cellular biology [7] .Further, concurrent developments in instrumentation such as advanced microscopic techniques and computerized image analysis methods [10] have led to the development of powerful tools for the design and execution of phenotypic assays. These advances have enabled novel assays that range from cell-based assays to assays based on whole animals. On the cellular front, advances in tissue culture technologies have permitted the creation of novel screens using primary cells, derived from patients in some cases, and sometimes by seeding them in a 3-dimensional matrix which simulates their natural environment to various degrees [11,12]. Novel devices dubbed 'organ on chip' have been developed where cells from the desired organ are seeded on appropriate substrates with the necessary modifications and perfusion of the 'chip' to mimic the in vivo environment in the organ [13].

In other cases, cellular organoids have been developed [14]. As an example of the growth in cell-based PDD screens, a highly coordinated national level effort has been initiated in the US towards the development of human tissue chips as a collaborative effort between National Center for Advancing Translational Sciences (NCATS) of the National Institutes of Health (NIH), the Defense Advanced Research Project Agency (DARPA) and the Food and Drug Administration (FDA); the long-range goal is to develop and test tissue chips for safety testing and drug discovery to be validated in academic and industrial labs; similar efforts are in progress in Europe and Japan [13]. Such collaborations among the government, industry and academia bode well for the acceleration and adoption of organ chip-based PDD for drug discovery.

Two of the stellar developments, the discovery of pluripotent stem cells and the methods for artificially inducing pluripotency into non-stem cells such as fibroblasts resulting in induced pluripotent cells (iPSCs), have opened heretofore unimaginable avenues for PDD. Stem cells derived from normal humans and patients can be differentiated efficiently into those of the organs involved in the disease, providing ideal screening systems for PDD; for instance, fibroblasts derived from patient skin biopsies can be induced to become pluripotent and then be differentiated into neurons or cardiomyocytes, etc. This approach has been used to design assays for indication areas such as cardiology, neurology, toxicology, etc [15-17].

Extensive efforts using cell-based assays are now devoted to the discovery of new drugs as well as repurposing of existing drugs, both efficacious ones and the ones that failed to show efficacy in late clinical trials. The repurposing effort provides a faster path to new drugs because most of the compounds in this class have undergone human safety studies and hence can move to the clinic without the delays for the preclinical and clinical safety studies required of totally new molecular entities. Based on this rationale, an NCATS-industry partnership titled "Discovering New Therapeutic Uses for Existing Molecules" (https://ncats.nih.gov/ntu) where PDD plays a significant role has been launched with several of the major pharmaceutical companies as partners [18].

Moving to a higher level of organization and complexity, a variety of whole animals, generally known as 'model organisms' has also been employed in PDD. Model organisms ranging from bacteria through worms and fish to rodents have been used in novel ways as phenotypic screens [11-19]. The relevance and hence the appropriateness of an organism depends on the phylogenetic distance of the model organism from humans, and the nature of applications. Thus, while bacteria, fungi and worms are clearly the necessary choice for anti-infective PDD, for metabolic diseases such as cancer and diabetes it is desirable and wiser to use rodents because they share much higher genetic similarity with humans. Hence, while academic laboratories have used lower organisms such as the yeast and the worm for developing screening assays for some diseases, the pharmaceutical industry has restricted itself mainly to the use of vertebrates, notably mice, which is further facilitated by the availability of scores of transgenic and knock-out mouse lines, many of which were developed as disease models. However, as one uses more human relevant organism, the cost increases and throughput decreases. Nonetheless, lower organisms such as yeast, worms, flies, as well as fish are ideal for delineating gene function where homologs of human genes occur, and thus they help target identification for TDD [19].

Due to the availability of mouse models for a wide range of diseases, multiplexed screening platforms using mice have been developed despite the lower throughput and higher labor intensiveness. One such multiplex platform which combines approximately 40 disease models covering 14 broad of therapeutic indication areas has been successfully applied to drug repurposing effort [20]. Another repurposing effort uses a suite of automated behavioral assays that use computer vision to extract multiple (~2000) behavioral features from drug-treated mice or transgenic mouse disease models to generate a behavioral profile of a large set of known psychotropic drugs and candidate compounds. This information can be analyzed using bio informatics techniques to produce behavioral spectra of the prototype drugs and candidates,

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enabling drug repurposing through comparisons [21]. In addition, the inclusion of disease models would further expand the power of this approach by interrogating compounds for the reversal of disease phenotypes.

Conclusion

Thus, in conclusion, although TDD displaced PDD from center stage of drug discovery since the 1980s, recent analyses have confirmed the power and utility of PDD to discover drugs on par with TDD. Given the renewed appreciation and enthusiasm for the value of PDD within in the drug discovery community and with the added impact of advances in cell culture, animal models and automated instrumentation, the future contribution of PDD to drug discovery appears bright.

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