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INTEGRATION OF SMALL MOLECULE DISCOVERY IN ACADEMIC BIOMEDICAL RESEARCH

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Abstract

Rapid advances in biomedical sciences in recent years have drastically accelerated the discovery of the molecular basis of human diseases. The great challenge is how to translate the newly acquired knowledge into new medicine for disease prevention and treatment. Drug discovery is a long and expensive process and the pharmaceutical industry has not been very successful at it despite its enormous resources and spending on the process. It is increasingly realized that academic biomedical research institutions ought to be engaged in early stage drug discovery, especially when it can be coupled to their basic research. To leverage the productivity of new drug development a substantial acceleration in validation of new therapeutic targets is required, which would require small molecules that can precisely control target functions in complex biological systems in a temporal and dose-dependent manner. In this review, we describe a process of integration of small molecule discovery and chemistry in academic biomedical research, which will ideally bring together the elements of innovative approaches to new molecular targets; existing basic and clinical research; screening infrastructure; and synthetic and medicinal chemistry to follow-up on small molecule hits. Such integration of multi-disciplinary resources and expertise will enable academic investigators to discover novel small molecules that are expected to facilitate their efforts in both mechanistic research and new drug target validation. More broadly academic drug discovery should contribute new entities to therapy for intractable human diseases especially for orphan diseases, and hopefully stimulate and synergize with the commercial sector.

Keywords

Small molecule; drug discovery; chemical screening; medicinal chemistry

The completion of human genome sequencing project has fundamentally changed the way that scientific research is conducted today. The genetic information of the human genome holds the key to our most fundamental questions in human biology of health and disease. However, despite the rapidly accumulating knowledge defining the molecular basis of human disease and the surge in the number of new potential therapeutic targets, annual new drug approvals by the US FDA have been steadily decreasing in the last decade to less than 20 new drugs in 2008–2009.

It is well known that drug discovery is a long and very expensive process (on an average of 15+ years and more than \$1 billion), and that the pharmaceutical industry has not been

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always successful at it despite its enormous resources and spending on the process. Many failures occur in the later stages of the drug discovery process that are due to lack of efficacy or intolerable toxicity in humans, making such failures a very high cost. Therefore, to leverage the productivity of new drug development a substantial acceleration in validation of potential therapeutic targets is required. Target validation, however, requires tremendous efforts in basic and mechanism-driven research at the molecular and cellular levels as well as in animal models. It has become increasingly realized that academic medical research institutions ought to be engaged in early stage drug discovery, especially when it can be coupled to the basic research activities [1–3].

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Ideally, validation of potential new drug targets would also benefit enormously from sustained interactions between academic laboratories and the pharmaceutical industry. Unfortunately, because of the high risk nature, and the extent of the basic mechanistic research involved, the pharmaceutical companies are generally not enthusiastic about an early-stage target validation, nor does a typical academic institution have access to high-throughput screening capabilities to routinely obtain commercially viable lead compounds for proof-of-principle target validation. Consequently, some major biomedical research institutions in the country in the recent years have started developing academic drug discovery programs. These programs aim to facilitate the identification of small molecule chemical compounds to be used for validation of potential new therapeutic targets being discovered in basic research. In addition, these small molecule agents that are capable of modulating the biological activity of a disease relevant protein, enzyme or macromolecule can also serve as powerful tools for research that are complementary to other technologies such as site-directed mutagenesis and gene knockdown by RNA interference.

The advantages of perturbing biological functions of macromolecular protein targets by using small molecules as chemical probes as compared to the other techniques result from the ability of small molecules to modulate selectively individual functions of a target protein in its endogenous form under physiological or pathophysiological conditions. Moreover, effects of such small molecule probes can often be controlled precisely in a temporal and dose-dependent manner, which is ideal as a research tool for investigating biological mechanisms, and certainly for validating potential new drug targets in complex biological systems.

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Therefore, small molecule discovery should not only facilitate early stage research in drug discovery through new target validation, but also advance mechanistic understanding of human biology of health and disease. Integration of small molecule discovery and chemistry is increasingly possible in academic research and below we outline some of the opportunities and challenges in this process.

CHALLENGES AND OPPORTUNITIES FOR TARGETING CELLULAR PROTEINS

Given the rapid advances in our understanding of structure-function of protein families in the human genome in general and the available technologies to gain specific knowledge for

a given protein target, it has become apparent that druggable [4,5] biological targets could include those beyond the usual classes such as enzymes and membrane-bound receptors, which have been the traditional focus of the pharmaceutical industry. New target classes include protein modular domains that engage in protein-protein interactions [6], which are modulated by post-translational modifications of amino acids. Examples are the bromodomains and chromodomains, which are present in chromatin modifying enzymes and associated proteins, and function to recognize acetylated and methylated lysine residues, respectively, in DNA-packing histones and transcriptional proteins during gene transcription [7,8]. Small molecule targeting of such protein interaction domains have advantages as compared to that of enzymes since these protein domains, which serve as functional units within large multi-domain proteins, exert their activity by interacting with their binding partners with high specificity but not necessarily high affinity individually (K_d of 1–100 μ M) [9–11]. This unique feature of these protein domains allows rapidly reversible and dynamic regulation of cellular biology such as signal transduction and gene transcription [12]. Therefore, targeting of amino acid modification-mediated protein interactions with selective, high affinity small molecules provides a new mechanism for modulation of biological activities and pathways [13–15].

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Because of the dynamic nature of protein-protein interactions in that target protein-biological ligand binding interface is not pre-formed in the absence of the ligand, effective lead discovery would require a screening assay, in which the target protein exists in a state mimicking ligand-bound state. As such, target structure-guided screening is a more effective and rational approach to ligand design, as it takes advantage of three-dimensional structure of the target, including the structure of the crucial functional site(s), which can guide one to build specific chemical entities that interact with these sites [16–19]. The novel ligands can be built with chemical entities constructed from building blocks identified from target structure-based screening and optimization coupled with focused library synthesis. The resulting linked compounds with high affinity and selectivity are then subject to detailed structure-activity relationship analysis of their interactions with the target by using NMR spectroscopy, X-ray crystallography, mass spectrometry and computational methods. Refinement, chemical diversification through chemical linkages and selectivity enhancement can be further achieved at this stage.

CHEMICAL SPACE FOR SMALL MOLECULE DISCOVERY

It has been estimated that theoretically as many as 10^{60} different chemical molecules could be in existence [20], highlighting the vastness of chemical space that is matched only to that of the cosmological universe. However, not all theoretic chemical species could be made due to the limits of current synthetic chemistry technologies; nor are they all necessary for identifying small molecules for a given biological target. In fact, studies have suggested that biologically active compounds, which have favorable physicochemical properties for interactions with biological molecules such as proteins and nucleic acids, are sparsely clustered in the chemical universe according to the target families. Therefore, it is thought that an ideal chemical library to be used for chemical screening would consist of small molecules that represent these discrete regions of biologically relevant chemical space. Chemical compounds could then be selected from a large collection of available chemical compounds using molecular descriptors such as pharmacophores, which describe key spatial arrangement and physicochemical properties related to their potential biological activity. Therefore, an ideal chemical library to be used for high-throughput screening should consist of small molecules with chemical diversity covering biological target families, as well as

drug-like physicochemical properties (see also below). Chemical hits and lead compounds identified from high-throughput chemical library screening can then serve as starting points for target-based medicinal chemistry that will develop these lead compounds into potent and selective chemical probes for a given target (see Figure 1).

Integration of discovery and biological applications of small molecule probes can enhance the development of biologically active compounds.

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It can also enable researchers to effectively investigate the underlying molecular mechanisms of important cellular processes in human biology and disease, and as well help discover and validate new therapeutic targets for disease treatment [20,21]. Given that the academic small molecule discovery is often aimed to achieve objectives of both aiding mechanistic research and validation of new therapeutic targets, the process can be different from the traditional linear drug discovery process. In the latter case, potential new drug targets are known based on the knowledge of a given disease condition. Compounds in a chemical library are evaluated in a high-throughput assay for their ability to interact with and functionally modulate the protein target. Initial chemical hits, which exhibit activity above a defined threshold, are further optimized for their activity through chemical modifications to yield chemical leads that possess the required pharmacokinetic properties. These leads are subsequently evaluated in *in vivo* biological and disease models and undergo lead optimization through additional chemical modifications. The latter process is performed in an iterative fashion, developing the lead into clinical drug candidates that are finally evaluated in human clinical trials before subjecting to the FDA review and approval as a new therapeutic agent for disease treatment.

Given that basic research of interest may or may not be linked directly to a particular disease model, chemical screening can still be conducted to identify small molecules that elicit specific phenotypic effects such as cell cycle arrest or apoptosis, or modulate *in vitro* functions of a target protein of interest. Such chemical agents can be used as research probes in chemical genetics research to identify biological targets responsible for the phenotypic effects, or validate specific proteins as potential therapeutic targets for disease treatment. Alternatively, when the chemical screening is conducted using different disease models, emerging small molecule chemical agents could be used to dissect the underlying molecular mechanisms of the disease models and also lay the foundation for further development of new therapeutic agent.

HIT FINDING AND HIGH-THROUGHPUT SCREENING

Starting compounds for the small molecule drug discovery process are conventionally termed “hits”; these are compounds with defined, reproducible activity in a primary, often biochemical (e.g. receptor binding, enzyme inhibition), screening assay (Figure 1). There are several approaches to hit finding [22–24] including de novo structure-based design, use of peptides or other endogenous ligands and screening approaches. These are not mutually exclusive and may be pursued in parallel depending on the maturity of a project or target area. Screening approaches are of interest because of their general applicability to all types of target at a very early stage. Since at least the early 1990s the use of high throughput screening (HTS) has been a standard component in the hit finding process in the pharmaceutical industry, where high throughput conventionally refers to testing of several tens of thousands (or more) compounds in the course of a few weeks or months.

Two elements are required for HTS, firstly a compound collection to screen and secondly assays suitable for high throughput and the infrastructure and/or hardware to implement them. In recent years both of these elements have become accessible to academic research institutions. Partly in response to the demands of large pharma (and the economic possibilities afforded by outsourcing) it has become possible for academic institutions to assemble significant screening collections (>100,000 compounds) from commercial sources (e.g. ChemBridge, ChemDiv, and MayBridge). The Mount Sinai collection currently comprises approximately 115,000 compounds pre-screened for physicochemical properties appropriate for drug discovery [25,26], for example Lipinski's "rule of five" [27]. The "rule of five" [27] was established based on an analysis of physicochemical properties of drug molecules which reached phase II clinical trials. The rule states that the drug-like properties of a chemical compound should consist of (1) less than five hydrogen bond donors (sum of OHs and NHs); (2) molecular weight is less than 500 Dalton; (3) LogP (the partition ratio, a measure of hydrophobicity) is less than 5; (4) less than ten hydrogen bond acceptors (sum of N and O atoms); and (5) compound classes that are substrates for biological transporters are exception to the rule [27].

Several further points are worth noting. Firstly a screening collection is not a static entity but may evolve in several ways including addition (either by purchase or in house synthesis) of focused sets of compounds directed at particular target classes, for example kinases, nuclear hormone receptors (NHRs) or G-protein coupled receptors (GPCRs). Libraries built around pharmacologically privileged scaffolds [28] natural product like [29] libraries maybe added. Secondly the collection will evolve naturally as compounds are added from Mount Sinai hit to lead and lead optimization programs (see below) and this provides significant opportunities since proprietary structural motifs exploited versus one target may be of use to discover hits in an unrelated area. One particular opportunity afforded by an in house screening collection coupled to active, ongoing research programs is the use of *in silico* docking methods to prioritize and focus screening (where there is sufficient structural information about targets) [30].

The second element in mounting an HTS campaign are assays suitable for high throughput and the infrastructure necessary to run them. *In vitro* assays emerging from laboratories doing basic biological research often involve multiple reagent additions, transfer steps, washes or filtrations. This type of protocol, while acceptable for daily or weekly evaluation of a handful of compounds, is not practical for HTS where on the order of hundreds of thousands of assay points will be required to complete the survey of even a modestly sized compound collection. The optimal assay strategy for HTS is a "mix and read" format in which biological components can be combined in microtiter plates, reagents added in one or two steps, then a signal read from the assay plate directly without filtrations, washes or separations. Again partly in response to the HTS demands of large pharma a wide variety of assay types are now possible in a mix and read format including receptor binding assays, protein-protein binding, enzyme assays and cell signaling assays [31]. Readouts are conventionally based on fluorescence, fluorescence polarization, bioluminescence or chemiluminescence with a variety of commercial sources for reagent. The robotics systems, liquid handling and plate readers for conventional HTS are all within the reach of major academic research institutions.

In addition to in-house compound collections and screening facilities, academic researchers may also access the Molecular Library Screening Centers Network (MLSCN, part of the NIH roadmap initiative) in which an assay is transferred to the MLSCN and screened versus a library of approximately 150,000 diverse compounds and assay results are then deposited in PubChem. Entry for assays to MLSCN is via a peer review process [32].

Beyond screening for single biomolecular interactions or transformations an emerging technique is the use of cellular imaging in high throughput mode in what has been termed high content screening (HCS) [33,34]. Here whole cells are imaged in tandem with screening compound challenge which allows simultaneous read out of multiple parameters, for example binding, downstream events relating to mechanism action (e.g. translocation of transcription factors) and markers of cell viability which an early indication of potential toxicity.

HIT TO LEAD AND MEDICINAL CHEMISTRY

The result of an HTS campaign is typically a small group of structures, which show activity in the primary screening assay. At this stage synthetic and medicinal chemists can begin to interact with their fellow scientists in biology and pharmacology in the interactive process of taking a screening hit to a medicinal chemistry lead and further to a clinical candidate. Academic centers committed to exploiting chemical biology to develop tools for basic research [35–37] and also aim to engage in drug discovery have setup chemistry groups dedicated to medicinal chemistry either along side their traditional academic chemistry departments, or de novo as at Mount Sinai. As mentioned above the drug discovery process is a collaborative enterprise requiring input from a range of scientific disciplines - indeed this is one of the organizational challenges for drug discovery in an academic environment which has been traditionally structured around research groups directed by individual investigators. Below we outline the early part of the compound optimization process from the perspective of progression of a compound from screening hit to medicinal chemistry lead – from a chemist's perspective.

Hit Confirmation

Data from an HTS campaign is typically triaged to eliminate false positives or artifacts, which might arise for example from interfering compound fluorescence in an fluorescence polarization (FP) assay [38] or compound protein aggregation artifacts [39,40]. Compound quantities in screening collections are generally small and often will be available only as a DMSO stock solution. Thus putative active structures are typically re-synthesized and scaled-up to confirm identity and purity. Hit compounds can then be made available to biologists in adequate quantity be interrogated in detail in the primary screening assay and other follow-up assays, often cell based, to confirm useful levels of activity. This is the first, simplest, but often crucial step in follow-up of an HTS campaign, or it might serve as the first experimental confirmation of an *in silico* (rational) design approach to hit finding.

Hit to Lead Chemistry

Confirmed hits are assessed and ranked based on a number of factors in addition to potency, particularly chemical tractability. A chemically tractable lead will be of reasonably low molecular weight structure (<500 Da), which is amenable to rapid analog synthesis to facilitate exploration of Structure Activity Relationships (SAR). Depending on the molecular type analogs may be available from commercial sources in which case they can be purchased. However generally, parallel libraries will be designed and synthesized to explore various sites for modification and improvement of the structure. This process may go through several cycles of parallel library synthesis and testing, with each iteration focusing and optimizing a feature or site on the hit structure. Several relatively new tools are available to medicinal chemists to facilitate rapid analog generation, for example microwave assisted synthesis [41], which allows reaction times to be shortened (and often gives fewer unwanted by products) and polymer assisted solution phase synthesis either as reagents or scavengers with simplifies reaction work-up and facilitates purification [42,43]. All or most of

the molecules from a hit to lead synthesis are likely to be suitable for inclusion in the broader screening library and so contribute to its growth and evolution.

A successful screening campaign will ideally yield three or four tractable lead series suitable for this type for early exploration. Compound series where potency and selectivity can be usefully modulated will be characterized in more detail with respect to physicochemical parameters such as solubility logP to confirm theoretical or calculated properties and confirm drug-like characteristics. In major academic research institutions it may be possible to form collaborations with established research groups for example in computational chemistry or structural biology for example to test theoretical models or aid in molecular design. This is the case at Mount Sinai where there is established expertise in computational biology, molecular simulation and experimental structure determination by using NMR and X-ray crystallography. A second institutional and organizational benefit is that medicinal chemistry might be applied to scientifically important projects in an academic setting where it may not be possible in the pharmaceutical industry for purely business reasons such as potential market size. Advanced molecules from tractable hit series where *in vitro* potency (in biochemical and cell based assays) and selectivity can be improved, serve as valuable research tools for interrogation of novel disease targets and may help validate novel or speculative biological mechanisms as potential drug targets – either of which may be a scientifically reasonable and successful end point for academic research (which is emphatically not the case in commercial drug discovery). Advanced structures from hit to lead chemistry may also for the jumping off point for further optimization.

Lead Optimization

Based on an assessment of biochemical potency, cell based activity, overall physicochemical profile one or more series of structures may be selected for further optimization. The objectives of the lead optimization are to increase potency in cell-based assays and to test molecules in more down stream *in vitro* and *in vivo* functional assays. *In vitro* ADME parameters such as microsomal stability and physical properties such as aqueous solubility will be monitored as leads progress [44]. The goal is an adequate balance of potency and physicochemical properties such that early rodent *in vivo* exposure data (PK) can be obtained. Early PK assessment has two goals, firstly to confirm that the series does indeed have potential for acceptable bioavailability, and, secondly to provide compound exposure data to help in the design and interpretation of initial *in vivo* efficacy experiments. In an academic setting where drug discovery targets are likely to be natural outgrowths and extensions of established basic research programs access to *in vivo* efficacy models, or in house expertise in setting them is likely to be an advantage. At the stage in lead optimization, where promising proof of concept *in vivo* efficacy data in animal models, broader pharmacological profiling of leads is appropriate: for example selectivity profiling *versus* panels of receptors and enzymes. This type of broad pharmacological profile is available from contract service organizations such as Cerep and Caliper Life Sciences. Early *in vitro* safety parameters may also be assessed, for example hERG channel activity (an indicator of potential cardiovascular liabilities) or CYP450 activity (an indicator of potential drug-drug interactions) [45]. This type of profiling can be reliably outsourced to contract research organizations, which currently service the pharmaceutical and biotech industries. Academic drug discovery projects may utilize the same type of resources, or for NIH funded projects resources such as the Molecular Pharmacology Research Program of the National Institute of Mental Health (NIMH). Lead profiling of this type will identify potentially problematic off-target activities, which can either be addressed with continued optimization to ameliorate or remove them (or result in a no-go decision on a lead series). Optimized leads with *in vivo* efficacy in animal models (either disease or biomarker), at an acceptable dose and route of administration, with no obvious toxicity or metabolic liabilities qualify as

candidates for more long term and detailed (including regulatory) animal toxicology studies and are sometimes designated as development candidates (DC) and conventionally regarded as the output of early drug discovery.

CONCLUSION

Integration of small molecule discovery and chemistry in academic biomedical research will ideally bring together the elements of (1) innovative approaches to new molecular targets (such as protein domain interactions); (2) existing basic and clinical research; (3) screening infrastructure; and (4) synthetic and medicinal chemistry to follow-up on small molecule hits. Such integration of multi-disciplinary resources and expertise will enable academic investigators to discover novel small molecules that are expected to facilitate their efforts in both mechanistic research and new drug target validation. More broadly academic drug discovery should contribute new entities for therapy (especially in neglected and orphan disease areas) and hopefully stimulate and synergize with the commercial sector.

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Figure 1. Schematic illustration of key steps involved in early stage small molecule drug discovery.