

Chemical Proteomics in Drug Discovery

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Abstract

Real-world drug discovery and development remains a notoriously unproductive and increasingly uneconomical process even in the Omics era. The dominating paradigm in the industry continues to be target-based drug design, with an increased perception of the role of signaling pathways in homeostasis and in disease. Since proteins represent the major type of drug targets, proteomics-based approaches, which study proteins under relatively physiological conditions, have great potential if they can be reduced to practice such that they successfully complement the arsenal of drug discovery techniques. This chapter discusses examples of drug discovery processes where chemical proteomics-based assays using native endogenous proteins should have substantial impact.

Key words: Chemical Proteomics, Drug target, Target deconvolution, Target validation, Drug discovery, Selectivity profiling

1. Introduction

Despite the dawn of the Omics era, drug discovery and development remains a notoriously unproductive and increasingly uneconomical process (1, 2). The dominating paradigm in the industry is still target-based drug design, with an increased perception of the role of signaling pathways in homeostasis and in disease (3). Because proteins represent the major type of drug targets, proteomics-based approaches, which allow to study a wide variety of proteins under relatively physiological conditions, have great potential if they can be reduced to practice such that they successfully complement the arsenal of drug discovery techniques (4). Industry standard assays of drug action typically assess the biochemical activity of the purified target protein in isolation. Frequently, recombinant enzymes or protein fragments are used instead of the full-length endogenous proteins. The activity of a

compound determined in this type of assays is often not predictive for its pharmacodynamic efficacy. One reason for this discrepancy is that an isolated recombinant protein, or protein fragment, does not necessarily reflect the native conformation and activity of the target in its physiological context, because of the lack of regulatory domains, expression of alternative splice variants, interacting regulatory proteins, or incorrect protein folding or posttranslational modifications. As a consequence, data generated in such assays may not be predictive for the effects of a compound or drug in cell-based or in vivo models. Ideally, assays should be developed to generate data on native proteins in cell extracts or cell fractions, under conditions carefully optimized to preserve protein integrity, folding, posttranslational modification state, and interactions with other proteins. Both activity-based and affinity-based chemical proteomics techniques, as described in this volume, should complement, or in some instances replace the traditional recombinant protein-based assays.

2. Chemical Proteomics Can Aid More Informed Selection and Validation of Targets

In target-based drug discovery, a project begins with the nomination of a target. The target is typically defined as a protein which should be

1. Tractable: Its biochemical activity can be modulated by the desired therapeutic agent (e.g., a small molecule) in a dose-dependent fashion.
2. Validated: It mediates a pathophysiological process such that its modulation reverses a disease-relevant parameter, which can be measured in disease-related cell-based or animal models, and is expected to be predictive of human disease.

Targets are often referred to as “druggable” and “clinically validated” when the modulation of the target was demonstrated to lead to the desired clinical outcome. Historically almost all druggable targets belong to a small number of target classes, biased toward cell surface proteins (e.g., G protein-coupled receptors, ion channels, or transporters) and a small number of intracellular protein classes (e.g., nuclear receptors, metabolic enzymes, kinases, or phosphodiesterases). A recent study estimated that the entirety of approved small molecule drugs acts through approximately 200 human proteins as targets (5), obviously a small number when compared to the 20,000–25,000 protein-coding genes in the human genome (6). It has been estimated that ten times as many suitable drug targets may exist, waiting to be discovered (7). In fact there are numerous proteins in pathways with a strong disease

implication, e.g., based on pathobiochemical and human genetic evidence, which are not tractable by current small molecule-based approaches. Chemical proteomics approaches should serve to expand the number of accessible drug targets by aiding the identification of tractable targets without the heavy bias toward the traditional target classes. This type of “target deconvolution” approaches was pioneered by the Schreiber laboratory in the classical studies which identified the molecular targets of immunosuppressants (8, 9). More recent exemplary approaches employed a combination of screening of diverse compound libraries in cell-based assays, which are not biased toward a particular family of targets, with chemoproteomics-based target identification. Huang et al. discovered the tankyrase proteins as tractable targets in the Wnt signaling pathway, which plays a central role in colon cancer but was characterized by a dearth of tractable drug targets (10). Using a related strategy, Fleischer et al. found that the potent and selective cytotoxic agent CB30865 exerts its effects by inhibition of nicotinamide phosphoribosyltransferase, an enzyme in the NAD biosynthetic pathway which helps cancer cells to sustain their increased energy metabolism (11). In another recent study, cell-based screening was performed for the upregulation of apolipoprotein AI production, and the proteomic profiling of hit compounds led to the unexpected discovery of bromodomain proteins as tractable targets for the modulation of the expression of apolipoprotein AI and certain proinflammatory genes (12). These bromodomain inhibitors exhibit a novel mechanism of action by blocking a protein–protein interaction formed between acetylated histones and BET-family bromodomains, which were not previously regarded as tractable targets. These and other successful studies support the notion that there is a general need for small molecules as research tools to study protein function, particularly for proteins which are not classical drug targets. Both the Structural Genomics Consortium (<http://www.thesgc.org>) and the Center for Protein Research (<http://www.cpr.ku.dk>) have recently initiated extensive programs for the development of chemical probes which will be made available to the scientific community.

3. Chemical Proteomics-Based Screening of Compound Libraries

Many drug discovery assays rely on the ability to express and purify the target protein in active form in the substantial amounts – typically milligrams of pure protein – necessary for the screening of compound libraries. The drug industry has encountered many so-called “difficult” target classes where this is not easily achieved, for instance, because the target protein is very large or requires additional factors like interacting proteins for proper activity. Therefore,

methods based on immobilized probe compounds to capture the target directly from a cell or tissue extract without further purification can represent a viable alternative strategy. This approach was used by Fadden et al. who captured purine-binding proteins from porcine tissue with ATP-derivatized Sepharose and performed affinity elutions with 5,000 different compounds, resulting in the identification of 463 small molecule compounds eluting a total of 77 distinct proteins. Among these, novel and structurally diverse inhibitors of the cancer target Hsp90 were identified, which were further optimized to enter clinical development (13). A different strategy was used by Bantscheff et al. who screened a compound library for histone deacetylase (HDAC) inhibitors in a human cell line extract, using an immobilized hydroxamate-based probe. Here, compounds were added directly to the cell extract rather than using them for elution, such that each compound was assayed for the inhibition of the binding of HDACs to the immobilized probe (14). An important feature of both approaches is that the entire complement of proteins binding selectively to the immobilized probe is screened simultaneously. This represents a major advantage over traditional screening approaches, in particular, for target classes with a substantial number of structurally related targets, like protein kinases or deacetylases, because possible “off-targets” (undesired additional proteins, which typically share a related active site with the target) are revealed early in the project. In conventional approaches, one is left to resort to educated guesses regarding possible “off-targets,” and distinct assays have to be configured for each individual protein.

4. Chemical Proteomics for Drug Target Profiling

Despite the fact that drugs are usually optimized against a single target, many compounds exhibit polypharmacology, i.e., they act on multiple targets. These “off-targets” can increase the therapeutic potential of a drug, but they might also cause toxic side effects, which represent a major reason why drugs fail in clinical development (15). An important recent example was the chemoproteomics-based identification of cereblon (CRBN) as a target of the drug thalidomide which mediates the drug’s teratogenic effects (16). However, for oncology drugs, polypharmacology is the rule rather than the exception, as they often target proteins from large target classes with a high degree of structural conservation around the active site, like protein and lipid kinases, HDACs, or heat shock proteins. Compared to a truly selective drug, such a spectrum of targets is more likely to produce toxic side effects, but in oncology the increase in therapeutic potential may outweigh this disadvantage (17). Conventional strategies typically rely on assay panels comprising 10–100 purified enzymes to address compound

potency, selectivity, and potential off-target liabilities (18). The recent progress in affinity-based proteomic techniques has enabled the direct determination of protein-binding profiles of small molecule drugs under close to physiological conditions. These techniques utilize immobilized compounds as noncovalent affinity baits (14, 19–22) or covalent active-site labeling probes (23, 24). The affinity probes are designed to selectively enrich a larger set of up to several hundreds of proteins defined by structurally related active sites, which can be viewed as chemically tractable subproteomes (25). Noncovalent probes are used either immobilized to an affinity matrix like sepharose or conjugated to biotin, and have been used successfully for purine-binding proteins (26), protein kinases including transmembrane receptor kinases (21, 22), lipid kinases (27, 28), phosphodiesterases (29), and HDACs (14). Covalent active-site labeling probes are typically biotin conjugates and have been applied to kinases (30), GTPases (31), methylases (32), dehydrogenases (33), serine-, cysteine-, metallo-, and proteasomal proteases (23, 34, 35), and HDACs (36). These methodologies typically generate protein affinity profiles for the immobilized compounds, which may reveal novel target candidates, but precautions must be taken to avoid false positives due to the background problems caused by nonspecific interactions with abundant proteins. Moreover, for the application to drug discovery, e.g., in screening or affinity/selectivity profiling assays, the generation of robust quantitative data for hit and lead compounds is an absolute necessity. These problems can be managed if the affinity capture protocols are formatted as quantitative competition-binding assays. This can be achieved by adding the compound of interest in its free form in the tissue extract, before or together with the affinity matrix or the active site label, such that the free compound binds to its targets in the lysate, thereby effectively competing with the capturing probe. By assaying the free compound in the cell extract over a range of concentrations, dose–response binding curves are generated for as many proteins as can be captured by the probe compound and robustly quantified. In case of the “Kinobeads” matrix for protein kinases and the hydroxamate matrix for HDACs developed by Bantscheff et al., more than 1,000 proteins were found to bind to the matrix and were routinely quantified in drug-profiling experiments using a competition binding assay format coupled to protein quantification by isobaric tagging and high-resolution LC-MS/MS peptide sequencing (14, 22). For a more detailed discussion of qualitative and quantitative small molecule target profiling, the reader is referred to recent comprehensive reviews (20, 23, 37). Finally, in addition to the *in vitro* applications described above, many chemical proteomics strategies can potentially be adapted to the identification and activity profiling of targets in living cells and in animal models (38).

In conclusion, the recent advances in chemical proteomics and in analytical instrumentation have promoted new drug discovery

strategies based on assays with increased content and better appreciation of the molecular context of the targets. These methodologies are providing complementary approaches to drug screening, drug target identification, and selectivity profiling, and have the potential to substantially contribute to in vivo studies and clinical studies of drug–target interactions.

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