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Target identification of natural and traditional medicines with quantitative chemical proteomics approaches

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ABSTRACT

Natural and traditional medicines, being a great source of drugs and drug leads, have regained wide interests due to the limited success of high-throughput screening of compound libraries in the past few decades and the recent technology advancement. Many drugs/bioactive compounds exert their functions through interaction with their protein targets, with more and more drugs showing their ability to target multiple proteins, thus target identification has an important role in drug discovery and biomedical research fields. Identifying drug targets not only furthers the understanding of the mechanism of action (MOA) of a drug but also reveals its potential therapeutic applications and adverse side effects. Chemical proteomics makes use of affinity chromatography approaches coupled with mass spectrometry to systematically identify small molecule–protein interactions. Although traditional affinity-based chemical proteomics approaches have made great progress in the identification of cellular targets and elucidation of MOAs of many bioactive molecules, nonspecific binding remains a major issue which may reduce the accuracy of target identification and may hamper the drug development process. Recently, quantitative proteomics approaches, namely, metabolic labeling, chemical labeling, or label-free approaches, have been implemented in target identification to overcome such limitations. In this review, we will summarize and discuss the recent advances in the application of various quantitative chemical proteomics approaches for the identification of targets of natural and traditional medicines.

Contents

Abbreviations: Akt, protein kinase B; CSNK1G3, casein kinase 1, gamma 3; DMSO, dimethyl sulfoxide; ICAT, isotope-coded affinity tag; iTRAQ, isobaric tag for relative and absolute quantitation; LDH, lactate dehydrogenase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NHS, N-hydroxysuccinimide; SAMS, S-adenosylmethionine synthetase enzyme; SILAC, stable isotope labeling by amino acids in cell culture; SpdSyn, spermidine synthase; TAMRA, carboxytetramethylrhodamine; SWATH, sequential window acquisition of all theoretical fragment ion spectra; TMT, tandem mass tags; OAT, ornithine aminotransferase; TCTP, translationally controlled tumour protein; PfATP6, P-type calcium transporting ATPase; PyrK, pyruvate kinase.

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1. Introduction

The current process of drug discovery from lead discovery to marketing of a new drug is tedious and costly, particularly due to unforeseen side effects of drug candidates during clinical trials or even after being marketed (Brown, 2007). For thousands of years, nature has provided us with a great source of bioactive molecules with medicinal value to treat many diseases (Böttcher et al., 2010; Carlson, 2010). Besides the need for isolation, structural determination, and synthesis of the naturally derived bioactive molecules, identifying their molecular targets and mechanism of action (MOA) is the focal point of interest in current natural product research. Many studies have focused on developing more efficient ways to reduce the time and cost for drug discovery efforts through systematic designing of new natural product analogs. This is typically followed by identification of their drug target profiles, which allows for the thorough exploitation of their therapeutic potential and minimizes their adverse side effects.

Although affinity purification (utilizing immobilized or biotinylated small molecules) and activity-based protein profiling (ABPP) have been widely used to study the targets of bioactive molecules in recent years, these methods generally suffer from the issue of nonspecific binding. Recently, quantitative proteomics approaches were introduced to overcome the specificity problem, and they greatly increased the accuracy of target identifications. Various approaches for target identification have been extensively reviewed in great details (Böttcher et al., 2010; Gersch et al., 2012; Schenone et al., 2013; Su et al., 2013; Ziegler, et al., 2013; Ursu & Waldmann, 2015). In this review, we will focus on the recent progress in the studies of natural and traditional medicines which combined quantitative proteomics and chemical biology approaches to identify their specific targets and elucidate their MOAs.

2. Drug target identification and chemical proteomics

Many drugs exert pharmacological effects by interacting with their protein targets. Identification of the specific protein target(s) of a drug is a critical step in unraveling its MOA, thereby enhancing our understanding of the pharmacodynamics of the drug, and allowing us to refine its future clinical application. In many cases, drugs have multiple protein targets, and identification of the complete target spectrum of a drug provides the most valuable information on the so-called "off-targets" of the drug, which are unexpected protein targets that may lead to unwanted biological alterations and toxicity. Furthermore, many drugs exert their disease modulation effects by simultaneously targeting multiple proteins, overthrowing the original concept of "one gene, one drug, one disease" that many researchers have adopted for decades (Lounkine et al., 2012), and the concept of "polypharmacology" is recently gaining traction.

Chemical proteomics is a multidisciplinary approach which integrates chemical synthesis with cell biology and mass spectrometry (MS). It provides a direct and unbiased platform for comprehensive profiling of targets of a given drug or biologically active natural product, and the proteomics output typically provides the most reliable data set for investigating and explaining the MOA of the agent. The most widely used approach to identify protein targets of a drug utilizes affinity purification coupled with mass spectrometry (MS) (Fig. 1) (Brown et al., 1994; Harding et al., 1989; Ziegler et al., 2013). In this method, the bead-immobilized or biotinylated drugs are firstly incubated with protein extracts before extensive buffer washes to remove noninteractive proteins. Subsequently, the protein targets are released by introducing high amounts of the free drug or via heat denaturation. Eventually, the bound proteins are identified using MS-based proteomics approaches (Fig. 1). Using these approaches, targets of several important drugs and their MOAs have been successfully revealed (Böttcher et al., 2010; Su et al., 2013; Ziegler et al., 2013).

Although powerful, the above methods can only be applied to cell lysates due to the requirement of a solid support or bulky tags (e.g., biotin tag) that preclude cell-based experiments. The *in vitro* target profiling may not accurately reflect the drug's actions in the *in vivo* physiological environment. To overcome this limitation, several groups have used the activity-based protein profiling (ABPP) approach combined with bioorthogonal click chemistry (Sletten & Bertozzi, 2009; Zhang et al., 2014) to identify drug targets both *in vitro* and *in situ* (Fig. 2) (Ovaa et al., 2003; Speers et al., 2003; Evans & Cravatt, 2006; Böttcher & Sieber, 2008; Fonović & Bogyo, 2008; Paulick & Bogyo, 2008; Böttcher et al., 2010; Nomura et al., 2010; Yang et al., 2010; Willems et al., 2011; Gersch et al., 2012; Liu et al., 2012; Shi et al., 2012). Activity-based probes (ABPs) covalently react with target proteins through their reactive drug moieties or a photoreactive group incorporated into the probes. The probe-labeled protein targets are then affinity-purified and identified by MS. With the increasing sensitivity of modern MS platform, even low-abundant protein targets can be successfully identified (Wang et al., 2015a).

3. The nonspecific binding in target identification

The basic principle of affinity purification and ABPP is straightforward (Figs. 1 and 2). Following the probe-target engagement, the specific protein targets and nonspecific binding proteins are separated by extensive buffer washing. However, a caveat of this approach is that no buffer washing is perfect and proteins that bind nonspecifically to a probe and/or a matrix often lead to false positives. Since there are numerous different proteins that exist in the cells and their biophysical properties and abundances vary (Rix & Superti-Furga, 2009; Zheng et al., 2015), nonspecific interactions are virtually impossible to eliminate. Nonspecific binding proteins increase the complexity of the samples to be analyzed by MS and are often difficult to differentiate from true protein targets. Therefore, negative control experiments are necessary in order to distinguish these nonspecific interactions from true interactions. Unfortunately, suitable negative control probe analogs are often unavailable (Rix & Superti-Furga, 2009). In addition, due to high sensitivity of MS, real targets may also appear in the list of proteins identified with the control pull-down as a result of nonspecific bindings. Commonly identified nonspecifically binding protein classes using traditional affinity-based approaches for the target identification are listed in Table 1 (Gao et al., 2012; Mellacheruvu et al., 2013; Trinkle-Mulcahy et al., 2008).

4. Quantitative chemical proteomics

The nonspecific binding problem of conventional chemical proteomics approaches can be largely overcome by quantitative proteomics. For relative quantification of proteins or peptides from different populations (e.g., samples with different concentrations of probe treatments, probe *vs.* controls, etc.), several stable isotope tagging methods are available. Stable isotopes of a given element have virtually identical physicochemical properties as the natural isotopes; therefore, the stable isotope incorporated proteins are almost identical to their natural counterparts. In practice, isotope labeling can be achieved by metabolic incorporation or chemical tagging. The difference in peptide masses