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Enzyme Annotation with Chemical Tools

Defining the activity of an enzyme in a cell-based system is preferable to in vitro biochemical techniques requiring extensive purification. Chiang et al. [1] use small molecules, RNA interference, and metabolomics to characterize a novel enzyme upregulated in cancer cells.

Traditional in vitro biochemistry is a powerful method for the analysis of protein function. However, such experiments often require purification and reconstitution steps that are difficult and time consuming. Additionally, as many proteins undergo posttranslational modifications and/or are involved in large protein complexes, the results of in vitro assays may be difficult to translate to in vivo systems. Cell-based methods avoid these problems as proteins are analyzed in a near-native system. Examples of such techniques include the use of small molecules, RNA interference (RNAi), and metabolic profiling. In this issue of *Chemistry & Biology*, Chiang et al. use these techniques to characterize the function and substrate of an enzyme in a near-native cellular system [1]. Chiang et al. and previous studies by Cravatt and colleagues [2, 3, 4] also use a relatively new technique, activity-based protein profiling (ABPP), in order to annotate the function and substrate of a novel integral membrane hydrolase, KIAA1363, upregulated in cancer cells.

ABPP uses chemical probes directed against specific enzyme families in order to profile altered enzyme activities in different contexts and to identify novel enzymes [5, 6]. There are three parts to an ABPP probe: (1) a reactive group that can covalently bind to the active site of an enzyme family, (2) a linker region, and (3) a chemical tag used to isolate the labeled enzymes [6]. Once cell extracts are treated with an ABPP probe, active enzymes that have bound and reacted with the probe can be identified in a gel analysis. One major benefit of this technique is that ABPP measures enzyme activity, rather than just monitoring protein abundance, which may or may not represent activity. Additionally, enzymes are monitored in cell extracts, avoiding the necessity for protein purification and the development of enzyme-substrate assays. Cell extracts are used, not whole cells, as the probes are often too large to go though the cell membrane. This can be overcome with tag-free probes allowing for the use of intact cells [7]. Furthermore, in an extension of the technique, termed competitive ABPP, small molecules can be screened for inhibitory activity against the ABPP probe, leading to identification of potent and selective, cell-permeable inhibitors [4, 8]. Chiang et al. show that ABPP studies can lead to the development of a potent and selective inhibitor for an enzyme with an unknown function, normally a nearly impossible task.

Work leading to the current paper from the Cravatt lab began when ABPP was used to screen for serine hydrolase activity in different cancer cell lines (Figure 1) [3]. In this experiment, a novel integral membrane hydrolase, KIAA1363, was found to be upregulated in cancer cells. A previously identified fluorophosphonate ABPP probe, which was known to interact with the hydrolase family of enzymes [2], was used for competitive ABPP studies. This led to the identification of a set of reversible trifluoromethyl ketone (TFMK) inhibitors against the mouse ortholog of KIAA1363 [4]. Although the TFMK inhibitors were not active against the human ortholog of KIAA1363 in cells, these studies enabled Chiang et al. to synthesize a TFMK analog, AS115 [1]. AS115 was found to inhibit KIAA1363 with an IC_{50} of 150 nM in cells. AS115 was subsequently used for cell-based studies of KIAA1363 in order to determine the substrate of the enzyme and to uncover its role in the ether lipid signaling pathway.

In traditional in vitro assays, identifying novel endogenous substrates for an enzyme can be a daunting challenge. The "discovery metabolite profiling" (DMP) method overcomes this issue by using liquid chromatography-mass spectrometry (LC-MS) to identify the substrates of an enzyme by analyzing the metabolites of cells in which the enzyme of interest is either active or inactive [9]. A small-molecule inhibitor is used to inactivate the enzyme; however, RNAi can theoretically be used if an active small-molecule inhibitor is not known. The main benefit of this technique is that it allows for the determination of endogenous substrates within a cell. The confounding problem is that secondary substrates may be identified; however, this could turn into an advantage, as these additional substrates may be helpful in defining the pathway in which the enzyme is involved.

To identify substrates of KIAA1363, Chiang et al. profiled cells with and without AS115, using LC-MS (Figure 1) [1]. They identified a difference in a set of lipophilic metabolites and subsequently confirmed them to be monoalkylglycerol ethers (MAGEs) by high resolution MS. This suggested that MAGEs are products of a KIAA1363-enzymatic reaction.

The biosynthesis of MAGE by the hydrolysis of 2-acetyl MAGE in the ether lipid pathway has previous been proposed [10, 11]. Chiang et al. were able to identify KIAA1363 as a missing enzyme in this pathway, converting 2-acetyl MAGE to MAGE. The use of the inhibitor AS115 and the comparison of cell lines varying in KIAA1363 expression levels were essential to the identification of KIAA1363 in this pathway.

As KIAA1363 activity is elevated in invasive cancers [3], Chiang et al. used RNAi to determine the effects of KIAA1363 depletion on cancer cells. They found that KIAA1363-knockdown cells had reduced tumor growth and impaired in vitro migration, compared to controls. Additionally, lower levels of MAGE and lysophospholipids were found in KIAA1363-knockdown cells, correlating with a decrease in KIAA1363 function. Thus, KIAA1363 contributes to cancer invasiveness through its regulation of the ether lipid metabolic pathway.

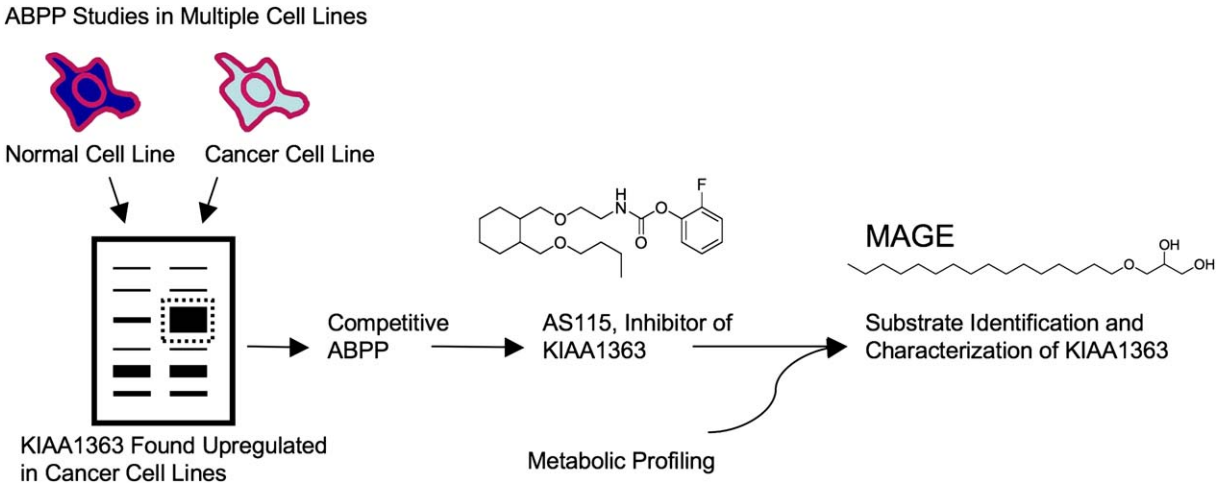

Figure 1. Scheme for Biochemical Characterization of KIAA1363 Using Activity-Based Protein Profiling and Metabolic Profiling

The use of ABPP identified a novel enzyme, KIAA1363, upregulated in cancer cells, but not in normal cells. Competitive ABPP facilitated the development of a small-molecule inhibitor of KIAA1363, AS115. Metabolic profiling was performed with AS115 to identify the endogenous substrate of KIAA1363. Further small-molecule studies with AS115 allowed for the biochemical annotation of KIAA1363 in a near-native system.

KIAA1363 is a model example displaying how ABPP can be used to classify cancer cells with different proteomic signatures. In these and many other cases, monitoring enzymatic activity may be more valuable than just monitoring protein expression levels. New protein targets for cancer therapeutics and new biomarkers for cancer can be identified in this manner. KIAA1363 was found to be upregulated in many cancers such as ovarian [1], breast [3], melanoma [3], and pancreatic cancer [12]. For KIAA1363, Chiang et al. were able to validate its potential use as a therapeutic target and as a new biomarker for cancer.

To date there are a limited number of "druggable targets" or proteins that can be selectively inhibited by small molecules. Competitive ABPP is a valuable approach for finding small-molecule inhibitors of enzymes, thereby increasing the number of druggable targets. This is especially true for enzymes of unknown function, as the specific function of the enzyme is typically necessary for small-molecule screening, but not for competitive ABPP studies. Expanding the use of small molecules to a wider range of proteins will have benefits for both basic research and disease treatment.

One final element of ABPP is the exciting prospect of chemical probe libraries targeting novel classes of enzymes. Enzyme classes with mechanistically defined active sites have enabled the development of ABPP probes, such as for the serine hydrolase family [2] and papain family of cysteine proteases [8]. However, the majority of enzyme classes have no known small-molecule scaffolds for the development of such probes. Moving to remedy this deficit, Adam et al. describe nondirected ABPP in which a specific library of reactive group probes was tested in order to broaden the ABPP technique to a wider group of enzymes [13]. To extend this further to other classes of enzymes, a more chemically and structurally diverse set of probe libraries must be constructed [6].

Ultimately, the combination of ABPP and metabolic profiling should allow for annotation of large numbers of enzymes with currently unknown functions. This advance will have a major impact on target discovery, target validation, and biomarker development.

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Selected Reading

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