Profiling Kinases using Chemoproteomics approach

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Overview

•Purpose: Profiling Kinases expression and activity in THP-1 cell lysates.

•Methods: Adenosine tri phosphate (ATP) activity-based probes were used to specifically label and enrich Nucleotide binding proteins for mass spectrometry analysis

•Results: Using ATP probes to assess Kinases activity, we have profiled a kinases inhibitor (Purvalanol B) activity. We have identified 1422 proteins among them more than 700 nucleotides binding sites were conclusively mapped by mass spectrometry.

Introduction

•Kinases and ATP-binding proteins have major roles in numerous key biological processes ranging from control of the cell cycle to signal transduction. Parasite ATP-binding proteins are therefore an attractive target for drug discovery. However the use of ATP mimetic or nucleotides binding site blocking agents as treatment can be have dramatic side effect. As kinases are evolutionary highly conserved, this lack of host–parasite specificity is due of high sequence similarity between host and parasite. It is therefore essential to develop an early warning system to identify "off" interactions at "omics" level (most completed catalog of ATP binding proteins).

•ABPP probe for kinases coupled at high resolution mass spectrometry seems be a promising tool for this purpose. The structure of these probes consists in a modified biotin (desthiobiotin) attached to the nucleotide through a labile acyl-phosphate bond. Acyl-phosphate bond is highly reactive with primary amine radical group of lysine. It covalently modifies this amino acid and induces a mass shift of + 192.1212 Da (See fig1).

Methods

•**Cells proteins labeling and profiling**: We have used 4E7 cells as starting material, cell were lyzed to extract proteins which were purified by size exclusion SPE to remove endogenous enzyme co-factors. The purified proteome was treated or not by Puralanol B and then labeled by ABPP. At this point two strategies were applied. For the first one, we captured labeled proteins by affinity chromatography and then digested them by FASP protocol. In this case all peptides from captured proteins are identified by mass spectrometry. For the second one we digested proteins first, using trypsin, and then we captured labeled peptides by affinity chromatography and analyzed them by mass spectrometry. In parallel we digested the whole cell proteome as control to evaluate the efficiency of enrichment by ABPP probe.

•**Mass spectrometry**: The peptide digest (100 ng) was loaded onto a nanoACQUITY UPLC Symmetry C18 Trap Column in trap and elute mode with ACQUITY UPLC Peptide BEH C18 nanoACQUITY Column. The run gradient was performed by Eksigent Ultra Plus nano-LC 2D HPLC (ABSciex, Framingham) system over 90 min with a gradient from 3% to 40% buffer B (buffer A: 0.1% formic acid; buffer B: 95% acetonitrile, 0.1% formic acid) at a flowrate of 300 nl/min. The Eksigent system was coupled to a TripleTOF® 5600 (ABSciex, Framingham) mass spectrometer. The acquisition parameters were as follows: for DDA mode one 250 ms MS scan (>30K resolution). Following each survey MS1 scan, MS/MS spectra for the 30 most abundant parent ions (m/z range 350-1250) were acquired (high sensitivity mode, >15K resolution). For DIA mode one 150 ms MS scan (>30K resolution), followed by 35 fixed SWATH windows each with a 75 ms accumulation time and a 350-1250 m/z range. MS/MS SWATH scans (high sensitivity mode, >15K resolution) were set at 26 amu window, Q1 isolation windows were covering entire mass range.

•**Data analysis**: DDA spectra processing and database searching was performed with ProteinPilot (v4.5 beta, ABSciex, Framingham) using Paragon and mascot algorithms. The search parameters were as follows: sample type: identification; cys alkylation: iodoacetamide; digestion: trypsin; instrument: TripleTOF 5600; special factors: Urea denaturation. ID focus: biological modifications with special variable modification Desthiobiotin label (+192.1212 Da on Lysine) . The database was downloaded from Uniprot (June 2015), filtering for reviewed mouse proteins only. The identification peak list was loaded into Peakview® (v2.0, ABSciex, Framingham) and Skyline. Peaks from SWATH runs were extracted with a peptide confidence threshold of 99% and a false discovery rate <1%. Label-free quantification was performed by using Marker View (v1.2.1, ABSciex, Framingham). The selection of the proper peak was performed using the automated assistance of PeakView. The absolute signal of peptide or protein was calculated by summing the extracted area of all unique fragment ions. Protein contents were compared using Student T-test, differences were considered as significant for p-values lower than 0.05.

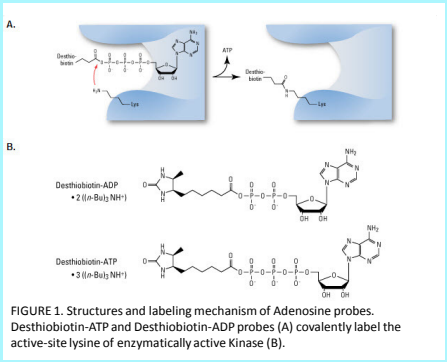

FIGURE 1. Structures and labeling mechanism of Adenosine probes. Desthiobiotin-ATP and Desthiobiotin-ADP probes (A) covalently label the active-site lysine of enzymatically active Kinase (B).

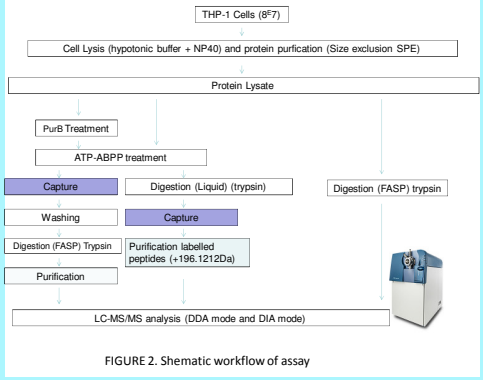

FIGURE 2. Shematic workflow of assay

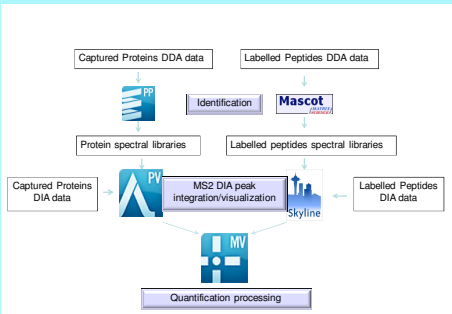

FIGURE 3. Schematic workflow of data processing

Results

•Using the MS Workflow (Figure 2), we have determined that more than 700 proteins were labeled. Among them 300 are reported as ATP-binding in mouse THP-1 cells tissues including CDK5. When comparing with unlabelled proteome, and efficient enrichment of ATP binding proteins was observed (See table 1). A specific inhibition of probe binding by inhibitor treatment was also observed for CDK5 (See fig 4).

•Analysis of labeled active sites showed a very good labeling efficiency (See fig 5), more than 95% of identified peptides were labeled by desthiobiotin.

Protein	p-value	ABPP	Total proteome	Fold Change
Tyrosine-protein kinase SYK	0,0002	1,47E+06	4,92E+05	2,98
Mitogen-activated protein kinase 14	0,0001	6,63E+05	1,56E+05	4,24
Cyclin-dependent kinase 1	0,0000	7,55E+05	3,17E+05	2,38
Serine/threonine-protein kinase 10	0,0002	7,47E+05	1,80E+05	4,14
Tyrosine-protein kinase CSK	0,0000	1,62E+06	3,85E+05	4,21
Mitogen-activated protein kinase 1	0,0002	4,52E+05	1,74E+05	2,59
Serine/threonine-protein kinase 38	0,0025	3,78E+05	1,23E+05	3,07
Adenylate kinase 2, mitochondrial	0,0009	7,19E+05	1,80E+05	3,99
[3-methyl-2-oxobutanoate dehydrogenase [lipoamide]] kinase, mitochondrial	0,0000	3,38E+05	8,35E+04	4,04
Serine/threonine-protein kinase VRK1	0,0376	3,26E+05	1,61E+05	2,02
Tyrosine-protein kinase Fes/Fps	0,0303	1,36E+05	6,37E+04	2,14
Cyclin-dependent kinase 5	0,0001	3,47E+05	6,11E+04	5,68

TABLE 1. Relative quantification of Kinases, ABPP vs no enrichment . Examples of enriched kinases.

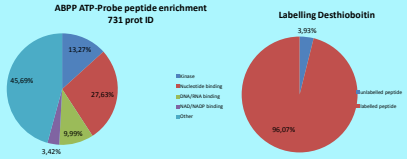

FIGURE 5. Repartition of ABPP enriched proteins by enzyme type . Labeling efficiency.

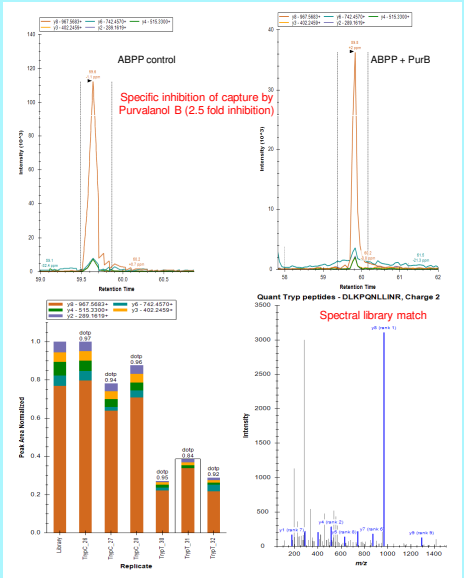

FIGURE 4. Example of CDK5 specific peptide (DLK[196.1]PQNLLINR) enriched by ABPP capture.
Upper right DIA XIC of control ; Upper Left DIA XIC of purvalanol B treatment. An ABPP probe binding inhibition was observed shown by a CDK5 decrease of activity induced by purvalanol B.

Conclusion

•ABPP ATP-Probe seems to be a potent tool to study ATP binding protein inhibitor effects at omics level. With a large number of identified proteins it is possible to observe on and off target interactions on very large data set of proteins.

•This type of assay are just one screwdriver of huge toolbox of chemoproteomics analysis as other ABPP probes are available, for instance GTP-desthiobiotin probe. Other ways for deciphering compound effect on biological model are worth to be investigated such as classical differential proteomics, or phosphosites analysis for early signal transduction.

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