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RESEARCH ARTICLE

PROTEOMICS

Tracking cancer drugs in living cells by thermal profiling of the proteome

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The thermal stability of proteins can be used to assess ligand binding in living cells. We have generalized this concept by determining the thermal profiles of more than 7000 proteins in human cells by means of mass spectrometry. Monitoring the effects of small-molecule ligands on the profiles delineated more than 50 targets for the kinase inhibitor staurosporine. We identified the heme biosynthesis enzyme ferrochelatase as a target of kinase inhibitors and suggest that its inhibition causes the phototoxicity observed with vemurafenib and alectinib. Thermal shifts were also observed for downstream effectors of drug treatment. In live cells, dasatinib induced shifts in BCR-ABL pathway proteins, including CRK/CRKL. Thermal proteome profiling provides an unbiased measure of drug-target engagement and facilitates identification of markers for drug efficacy and toxicity.

The complete determination of the proteomic state of a cell, referred to as the proteotype, links genotype and the cellular phenotype. Thus, the proteotype of the target cell or tissue represents an important context for the study of drug action. However, the task of accurately describing a proteotype remains daunting because of the inherent complexity of the proteome, comprising in excess of 10,000 gene products expressed at levels differing over six or more orders of magnitude, many of which occur in different splice isoforms and with different posttranslational modifications at different subcellular localizations (*1*). Current expression proteomics methods describe proteotypes as lists of proteins and semiquantitative expression levels (*2*). In addition, there are methods for the cell-wide assessment of some but not all post-translational modifications (*3*, *4*). Studies focused on the mechanism of bioactive compounds, such as small-molecule drugs or peptides, frequently rely on affinity-based enrichment strategies to identify prospective binding partners from a cell extract (*5*–*7*). These approaches can be combined for the differential study of cellular phenotypes—for instance, the status of signaling pathways or for biomarker discovery (*8*, *9*). However, unbiased approaches for the cell-wide assessment of protein state and protein function are not available.

Changes in the thermal stability of proteins are frequently used to study ligand binding (*10*, *11*) and, with the recent development of the cellular thermal shift assay (CETSA), can now be observed in living cells (*12*), enabling the monitoring of target engagement, which is a key parameter in drug discovery (*13*). We extended this approach to address two challenges critical in drug discovery: target and off-target identification and discovery of molecular biomarkers for drug efficacy. By combining the CETSA method with multiplexed quantitative mass spectrometry (MS), we established the proteome-wide determination of protein thermal stability in intact cells as an independent and complementary strategy for the characterization of cellular proteotypes. Our approach, termed "thermal proteome profiling," includes but is not limited to the monitoring of protein-ligand interactions directly in cells or tissues because in theory, any modification of a protein can affect its thermal stability (*14*). Here, we demonstrate that monitoring thermal stability across cellular proteomes in different states, such as under drug treatment, enables the identification of direct physical interaction partners and downstream effectors as markers of target engagement and drug efficacy.

Proteome-wide profiling of protein thermal stability

To monitor the thermal stability of proteins across 10 different temperatures, we used the recently developed neutron-encoded isobaric mass tagging reagents (TMT10) (*15*) in conjunction with high-resolution MS. This allowed acquisition of full melting curves for a large proportion of expressed soluble proteins in a single liquid chromatography–MS (LC-MS)/MS experiment. In a typical experiment, cells were cultured under differential conditions, such as drug treatment (Fig. 1). For each condition, the cells were divided into 10 aliquots, each of which was briefly heated to a different temperature followed by extraction with phosphate-buffered saline (PBS). Around their intrinsic melting temperature, proteins in the cell denature and subsequently aggregate (*16*), resulting in their gradual disappearance from the PBS-extracted samples with increasing temperatures (*12*, *17*). This protocol is only suitable for the soluble fraction of the proteome because membrane proteins are not solubilized under these conditions. After extraction, each sample was trypsinated and labeled with a different isotope-coded isobaric mass tag (*15*), and the 10 samples from each condition were mixed and analyzed by means of LC-MS/MS. The reporter ion intensities acquired in the MS/MS fragment spectra were used to fit a curve and calculate a specific melting temperature (T_m) for each protein, which was then compared between the vehicle-treated and drug-treated samples. The resulting curves that display the increase in protein aggregation with temperature in many cases directly reflect the underlying unfolding or "melting" event. For many proteins, a good correlation between the thermofluor unfolding assay and heat-induced precipitation in solution, or in cells by CETSA, has been demonstrated (*11*, *17*), suggesting that properly shaped curves represent real unfolding events. Hence, ligand-induced curve shifts indicate a change in the thermal stability of the protein rather than a change in the aggregation properties. In a variation of the cell-based protocol, thermal stability can also be assessed in a cell extract. This alternative strategy avoids the separate extraction of each cell sample, potentially allows more controlled conditions (*12*), and in conjunction with cell treatment experiments, enables distinguishing T_m shifts induced by ligand binding from those induced by downstream modifications.

The thermal profile of a cellular proteome

We acquired quantitative thermal stability data for 5299 proteins across 10 different temperatures from the human K562 chronic myeloid leukemia cell line. This data set could be regarded as the first description of the melting proteome ("meltome") of a human cell. Thermal profiles were acquired in two experimental settings in which either intact cells or cell extracts were heated. In both settings, we noted a weak but significant anticorrelation of the thermal stability with molecular weight because smaller proteins tend to be more stable (fig. S1). A comparison of the thermal stability across a set of 3204 proteins robustly quantified in both intact cells and cell extract revealed marked differences in melting properties between cells and extract (Fig. 2 and table S1). Hierarchical cluster analysis of the temperature-dependent relative protein concentrations in the heated cell samples revealed a group of proteins that exhibited an increase in concentration at ~50°C, followed by a pronounced decrease at ~56°C, and another group of proteins with a concentration increase at ~63°C.

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