

Large-scale Determination of Absolute Phosphorylation Stoichiometries in Human Cells by Motif-targeting Quantitative Proteomics

Protein phosphorylation is one of the most critical post-translational modifications for regulating cellular function. Measuring phosphorylation events on the proteome scale provides a system view of activated signaling pathways. In a pioneering study, an international research group led by Dr. Yu-Ju Chen from the Institute of Chemistry, Academia Sinica and Prof. Yasushi Ishihama from the Graduate School of Pharmaceutical Sciences, Kyoto University has developed a novel mass spectrometry-based technique for the first measurement of the basal level of phosphorylation stoichiometry in a single human phosphoproteome and identified potential molecular changes associated with resistance to the cancer drug gefitinib in lung cancer cells. The study was published online on March 27, 2015 in the journal *Nature Communications*.

Deregulated signaling through protein phosphorylation is intimately linked to the pathogenesis of human disease and is one of the most clinically accessed post-translational modifications for developing new therapeutic strategies. The signal-induced alteration in phosphoprotein is regulated either by upstream kinase/phosphatase activity causing changes in *phosphorylation stoichiometry*, defined as the ratio of the total amount of protein phosphorylated at a specific site to the total amount of protein, or by transcriptional regulation to modify protein abundance. However, the traditional quantitation approach can only measure relative quantitation of phosphorylation events without information about the absolute stoichiometry of modification within proteins; direct measurement of phosphorylation stoichiometry, which allows digitization of the cellular signaling network, still remains a challenge using existing approaches.

To overcome the current bottleneck in accessing the stoichiometry of single-state human phosphoproteome, we have developed a motif-targeting quantitative proteomic approach by integrating enzymatic kinase reaction and an isotope-based quantitative proteomic strategy. The quantitation accuracy and sensitivity of this approach was demonstrated on proof-of-concept experiments in lung cancer cells; phosphorylation stoichiometry of >1000 phosphorylation sites including 366 low abundant tyrosine phosphorylation sites were successfully measured with high reproducibility. To our knowledge, this approach reveals the first large-scale measurement of the basal level of phosphorylation stoichiometry in a single state human phosphoproteome.

This research group further applied this developed motif targeting quantitative approach for phosphorylation stoichiometry profiling of drug resistance/sensitive lung cancer cells. By comparison of tyrosine kinase inhibitor (TKI) sensitive (PC9) and resistant lung cancer cells (PC9/gef.), the quantitative information not only revealed that post-translational phosphorylation changes are significantly more dramatic than those at the protein as well as mRNA levels, but also

suggested potential drug-targeting proteins in the kinase-substrate network associated with acquired drug-resistance. We expect that this newly developed approach will have a wide range of applications providing system-wide maps of protein phosphorylation stoichiometry for either single or multiple cellular states under physiological or pathological regulation.

The complete article is available at the *Nature Communications* journal website at:

<http://www.nature.com/ncomms/index.html>.

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