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Short Communication

Role of Phosphoproteomics in Protein Activity

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ABSTRACT

Phosphoproteomics is a branch of proteomics that searches for, catalogues, and describes proteins with phosphate groups as a posttranslational alteration. Phosphorylation is an important reversible change that controls protein activity, subcellular localization, complex formation, protein degradation, and so cell signalling networks. With all of these findings, it's believed that 30% to 65% of all proteins are phosphorylated, with some being phosphorylated numerous times. In comparison to expression analysis, phosphoproteomics adds two more levels of data. Because a change in phosphorylation status usually invariably reflects a change in protein activity, it provides indications as to which protein or pathway may be activated. While phosphoproteomics will substantially increase our understanding of the amount and types of phosphoproteins, its most promising use is the quick investigation of complete phosphorylation-based signalling networks.

Keywords: Phosphoproteomics; Antiphosphotyrosine antibodies; Hyperphosphorylation

DESCRIPTION

Phosphoproteomic analyses are ideal for studying signalling network dynamics [1]. In one experiment, cells are labelled with SILAC and then activated with a specific growth factor. The cells are harvested at different times, and the lysates are mixed for tandem MS analysis. This enables researchers to monitor the phosphorylation status of a large number of phosphoproteins in the cell over time. This methodology is far more powerful than standard biochemical methods for investigating signalling network function because it can measure the global phosphorylation state of multiple proteins at many time points [2].

In one study, researchers were able to compare the phosphorylation states of 127 proteins in unstimulated and EphrinB1-stimulated cells. With EphrinB1 stimulation, 40 of the 127 proteins showed elevated phosphorylation. The researchers were able to create a signal transduction network for the proteins downstream of the EphB2 receptor by combining this knowledge with previously published data. A large-scale identification and quantification of phosphorylation events produced by the anti-diuretic hormone vasopressin in the kidney collecting duct was part of another recent phosphoproteomic investigation. The researchers discovered 714 phosphorylation sites on 223 different phosphoproteins, including three new phosphorylation sites in the vasopressin-sensitive water channel aquaporin-2.

It is undoubtedly possible to examine the whole complement of phosphorylated proteins in a cell. This is due to improvements in phosphoprotein and phosphopeptide enrichment strategies, chromatography-based fractionation techniques, and mass spectrometry-based approaches for selectively seeing phosphorylated residues [3]. Despite the fact that contemporary phosphoproteomic analysis methodologies have substantially improved, sample loss and discrepancies in sample preparation, enrichment, and equipment still exist. For high-throughput phosphoproteomic research, bioinformatics tools and biological sequence databases are also required.

Radioactive labelling with 32P-labeled ATP followed by SDS polyacrylamide gel electrophoresis or thin layer chromatography was previously used to isolate phosphorylated proteins [4]. Traditional approaches are ineffective because huge amounts of proteins required for phosphorylation analysis are impossible to get. As a result, affinity purification with phosphospecific antibodies, immobilised metal affinity chromatography, strong cation exchange chromatography, or titanium dioxide

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chromatography are the most common and straightforward methods for enriching phosphoproteins.

CONCLUSION

Cancer research has focused on alterations in the phosphoproteome during tumour development since the birth of phosphoproteomics. Phosphoproteins could be used as cancer indicators in diagnostics and therapy. In fact, research has revealed that breast and liver cancers have unique phosphotyrosine proteomes. In breast cancers, there is also evidence of hyperphosphorylation at tyrosine residues, but not in normal tissues. These findings show that the tumour phosphoproteome can be mined for new biomarkers. While phosphoproteomics has substantially increased our understanding of the amount and types of phosphoproteins as well as their roles in signalling networks, there are still some limits to these methods. To begin with, anti-phosphotyrosine antibodies do not differentiate between tyrosine-phosphorylated proteins and proteins associated with tyrosine-phosphorylated proteins when used to isolate them.

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