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I E C B

Chemical approaches to study protein fatty acylation

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Protein fatty acylation (FA) is the addition of a long chain fatty acid to Glycine, Cysteine, or Lysine residues of a protein. This post-translational modification (PTM) enables direct interaction with cell membranes, and regulates protein function and localization. N-myristoylation and S-palmitoylation are the most common FAs found in all Eukaryotes, and are important in normal function and disease. However, the diversity, abundance, and regulatory mechanisms of protein FA in vivo are not fully understood. In addition, the intricate interplay of these modifications in the disease context presents a challenge for drug development.

FA of proteins can be studied by metabolic tagging with small, 'clickable' chemical reporters that do not disrupt normal metabolism and function. Subsequent chemoselective reactions enable the selective addition of multifunctional labels to tagged fatty acylated proteins. A fluorophore label allows visualization of the fatty acylated proteins by in-gel fluorescence, while a biotin label allows for enrichment on streptavidin beads for global whole-proteome profiling of protein FA.

In the first study, this labeling technology was used to validate N-myristoyltransferase (NMT), the enzyme that adds myristoyl group to proteins, as a drug target in cancer therapy. A small library of inhibitors was screened, and their on-target activity was demonstrated in cells. N-myristoylated proteins in cancer cells were identified by quantitative chemical proteomics using a combination of the best inhibitor, and the clickable chemical reporter. NMT inhibition was found to induce ER stress, cell cycle arrest, and apoptosis in cancer cells.

The second study involved S-palmitoylation of proteins. S-palmitoylation remains more challenging to study due to its reversibility, the presence of multiple S-palmitoylating enzymes, and the lack of selective inhibitors. The labeling technology was used, in combination with other methods, to perform S-palmitoylation whole-proteome profiling, direct site identification, and lipid structure characterization. The function of S-palmitoylation of proteins involved in vesicular transport was also studied.

All these studies will ultimately help in understanding the functions and regulatory mechanisms of fatty-acylated proteins in physiology and disease.