Journal Club No. 817 December 18, 2018

蛍光標識を用いた小胞体プロテオームのケミカルプロファイリング

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たんぱく質の細胞内局在を知る場合、蛍光試薬や蛍光たんぱく質の発現を使って、顕微鏡下で観察する手法や、オルガネラの分画を用いて解析する手法が使われる。プロテオーム解析でオルガネラのたんぱく質を網羅的に解析するためには、分画法を用いて行われるが、手間も技術も必要となる。小胞体にターゲッティングする蛍光物質を、オルガネラのたんぱく質に結合させ、抗体を用いた分画後、解析することによって小胞体たんぱく質を網羅的に解析する技術が開発された。同様に他のオルガネラに局在する化合物を用いることによって、より簡便にオルガネラ間のたんぱく質の動きを網羅的にプロファイリングすることができるようになるかもしれない。

紹介論文

Chemical Profiling of the Endoplasmic Reticulum Proteome Using Designer Labeling Reagents.

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J Am Chem Soc. 2018 Nov 28. doi: 10.1021/jacs.8b08606. [Epub ahead of print]

要旨

The endoplasmic reticulum (ER) is an organelle that performs a variety of essential cellular functions via interactions with other organelles. Despite its important role, chemical tools for profiling the composition and dynamics of ER proteins remain very limited because of the labile nature of these proteins. Here, we developed ER-localizable reactive molecules (called ERMs) as tools for ER-focused chemical proteomics. ERMs can spontaneously localize in the ER of living cells and selectively label ER-associated proteins with a combined affinity and imaging tag, enabling tag-mediated ER protein enrichment and identification with liquid chromatography tandem mass spectrometry (LC-MS/MS). Using this method, we performed proteomic analysis of the ER of HeLa cells and newly assigned three proteins, namely, PAICS, TXNL1, and PPIA, as ER-associated proteins. The ERM probes could be used simultaneously with the nucleus- and mitochondria-localizable reactive molecules previously developed by our group, which enabled orthogonal organellar chemoproteomics in a single biological sample. Moreover, quantitative analysis of the dynamic changes in ER-associated proteins in response to tunicamycin-induced ER stress was performed by combining ER-specific labeling with SILAC (stable isotope labeling by amino acids in cell culture)-based quantitative MS technology. Our results demonstrated that ERM-based chemical proteomics provides a powerful tool for labeling and profiling ER-related proteins in living cells.