Dynamic Quantitative Analysis of the Nucleolar Proteome Using an Isobaric MassTag Thiol Approach

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Abstract

The nucleolus represents a large, highly dynamic multifunctional nuclear organelle. It plays a key role in ribosome biogenesis and participates directly or indirectly in cell-cycle regulation, senescence, proliferation, differentiation and maturation states. Recently, several studies have dramatically increased the scientific community's knowledge of nucleolar proteome regulation using mass-spectrometry-based quantitative analysis. Here, we present a dynamic analysis on the nucleolar proteome using Isobaric MassTag Thiol technology, a novel quantitative system based on a class of isobaric reagents and tandem mass spectrometry (MS). The properties of this technology allow analytes modified by the MassTag Thiol reagents, to be separated as a group from other molecules and distinguished from each other through tandem MS. Six nucleolar protein extracts, isolated from HeLa cells that were treated with the metabolic inhibitor actinomycin D for different time periods, were modified with a unique MassTag Thiol reagent on the free cysteine residues. Based on the MS results, a total of 542 proteins were qualitatively identified, and 232 proteins were unambiguously quantified. The quantification data demonstrate that the nucleolar proteome significantly changes over time in response to differences in growth conditions, which is consistence with the previous observations from several groups. The Isobaric MassTag Thiol approach should provide a widely applicable multiplexing tool in the quantitative proteomics field.