Insights from Redox Proteomics: Focus on S-glutathionylation Stoichiometry and Redox Status of Protein Cysteines

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Introduction

Reversible oxidative modifications of cysteines (Cys) can modulate the function of a protein in response to a change in the local environment, such as oxidative stress. These modifications are the main component of redox signaling and involved in regulation of various critical cellular processes under physiological and pathological conditions. The study of Cys modifications is facilitated by emerging quantitative proteomic approaches, which enable global identification of site-specific redox modifications in cells under stressed conditions. However, the occupancy of reversible oxidative modifications at a given Cys residue, which is important for understanding the functional role of the Cys modification, is usually overlooked. Herein, we focus on determining the occupancy of S-glutathionylation (SSG) and total reversbile oxidation on Cys.

Methods

Resin-assisted quantitative proteomics was used for site-specific measurement of SSG occupancy and redox status of protein cysteines in mouse macrophages. Total cysteines were measured after DTT reduction of all the cysteines in proteins. Total oxidized cysteines and SSG-modified cysteines were measured after N-ethylmaleimide (NEM) blocking followed by DTT and glutaredoxin reduction, respectively. The reduced proteins were captured on thiol-affinity resins, followed by on-resin digestion and tandem mass tag (TMT) labeling. The levels of total cysteines, total oxidized cysteines, and SSG-modified cysteines from the same sample were quantified using 6-plex TMT labeling. Four biological replicates were analyzed with two sets of TMT6 experiment using LC-MS/MS.

Preliminary Data

A total of 4099 unique Cys sites from 1959 proteins were confidently identified for quantitative analysis. The observed average oxidation level of protein Cys is ~11.9% in mouse macrophages, which is in agreement with the fact that the majority of proteins in cells are in a reducing status in vivo. The average SSG occupancy in total Cys is 4.0%; however, the average percentage of SSG in total Cys oxidation is 32.0%, supporting that SSG is one of the major oxidative modifications of Cys. We found the redox potential of a cellular organelle is well correlated with the level of Cys oxidation in the organelle. For example, endoplasmic reticulum and lysosome are two of most oxidizing organelles, in which the oxidation levels of Cys are 16.4% and 14.3%, respectively. These oxidation levels are much higher than those from reducing organelles, such as nucleus and mitochondrion, in which the oxidation level of Cys is only ~10%. However, the average SSG/total Cys oxidation ratio is relatively low in the oxidizing organelles, which suggests other form of oxidative modification, such as protein disulfide, becomes dominant at an increased oxidizing environment. In contrast, the reducing organelles have an average SSG/total Cys oxidation ratio larger than 32.0%, suggesting SSG is the dominant modification and plays important regulation role in these organelles. Moreover, comparing with our previous data, we found that the Cys residues that have lower basal oxidation typically have higher fold-changes than those with higher basal oxidation in response to stress stimuli, suggesting the selectivity of cellular redox response. A number of Cys sites with a high basal level of oxidation were also

identified as redox-sensitive sites. Most of these sites were involved in inhibition of protein activities after oxidation. Our result demonstrated the stoichiometry information of Cys modification is important for identifying redox-regulated proteins.