Targeted Ubiquitination of Glutathione Peroxidase 1

Cathy Tran
Lucia A. Seale, PhD, University of Hawaii, Pacific Biosciences Research Center
Kayla A. Hallam, PhD Candidate, Cell and Molecular Biology Program, John A. Burns School of Medicine, University of Hawaii

ABSTRACT

The selenoprotein glutathione peroxidase 1 (GPX1) protects cells from oxidative stress by catalyzing the reduction of harmful hydroperoxides into harmless alcohols and water, maintaining redox balance. To execute this antioxidant function, GPX1 levels must be regulated through protein synthesis and degradation. While the regulation of GPX1 synthesis is characterized, its degradation regulation remains unclear. To avoid excess accumulation in cells, GPX1 must undergo degradation, possibly through the ubiquitin-proteasome system. In this system, proteins are tagged with ubiquitin in specific lysine residues, allowing proteasomes to recognize and degrade them. We hypothesize that GPX1 is degraded through the ubiquitin-proteasome system. Alpha Fold 2.0 and mass spectrometry were used to predict lysine residues on GPX1 where ubiquitin could bind. After identifying lysine residues, Human Embryonic Kidney 293 (HEK293) cells were transfected with GPX1 mutated in specific lysine sites, preventing ubiquitin binding. Cells were treated with 20 µg/mL cycloheximide and 10 µM MG132 to inhibit protein synthesis and proteasomal degradation respectively, and cultured under low (20 nM) or adequate (80 nM) selenium levels. Total protein was extracted, quantified, and assessed for GPX1 through Western blots with a specific antibody. We confirm that GPX1 levels are directly regulated by selenium levels. Mutated GPX1 levels were further reduced when compared to wild-type GPX1 levels, under both low and adequate selenium levels, indicating that GPX1 may undergo degradation that is not dependent on ubiquitin and selenium. Our findings provide insight on how a degradation mechanism independent of selenium controls GPX1 levels and consequently, maintains redox balance.

KEYWORDS

Glutathione peroxidase 1, Ubiquitin-proteasome system, Selenoprotein

ACKNOWLEDGEMENTS

The STEP-UP HS program is supported by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health, Grant Number 5R25DK078386-20. NIDDK grant R01DK128390 provided support to the Seale Lab.