Chemical Proteomics Identifies Ketone Body-Mediated Cysteine Modifications Regulating Redox Function

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Abstract

All the studies of ketogenesis-dependent post-translational modifications (PTMs), notably those mediated by ketone bodies, β-hydroxybutyrate (Bhb) and acetoacetate (Acac), have focused on lysine acylations. However, given the chemically diverse and reactive nature of metabolites generated, it remains unclear whether non-lysine modifications can also happen. Here, we develop an acetoacetate-alkyne (Acac-alkyne) chemical probe that enables efficient metabolic labeling, robust fluorescent visualization, and site-specific identification of Acac-modified proteins. By combining chemical proteomics with open-search strategy, we showed that Acac induces previously uncharacterized cysteine modifications in LNCaP cells. Notably, cysteine crotonation (Ccr) is validated by employing both probe-based and standard peptide-based co-elution assays. Metabolic pathway tracing further identifies BDH1 and ECHS1 as key enzymes that generate Ccr formation. We further demonstrate that Ccr at PRDX3 C229 site impairs dimerization and redox activity, linking this newly discovered modification to the regulation of cellular reactive oxygen species. Together, these findings establish ketone metabolism as a novel source of cysteine modifications and provide an alternative mechanistic pathway to explain the profound biological effects of ketone bodies.

Funding Acknowledgements

his work was supported by the startup fund of Dr. Zhipeng Wang from the Desai Sethi Urology Institute (DSUI) and the Sylvester Comprehensive Cancer Center (SCCC) at the University of Miami Miller School of Medicine.

Conflicts of Interest Disclosure Statement

The author declares no conflicts of interest.