

Extended Abstract



Journal of Computational Methods in Molecular Design, 2021 https://www.scholarsresearchlibrary.com/journals/journal-of-computational-methods-in-molecular-design/

Oxygen-sensitive interactions between glycolytic enzymes and a cancer-testis antigen established signalling scaffold are regulated by lysine acetylation

Soren Naaby Hansen, Aalborg University Hospital, Denmark

E-mail: sonh@rn.dk

Reactivation of the male gametogenic expression program is tightly associated with the most malignant and metastasis-prone tumours and the emergence of aggressive sub clones of tumour cells, which are highly resistant to stress-induced apoptosis. While the cancer-testis antigens (CTAs) CABYR and AKAP3/4 roles during gamatogenesis and their importance for flagellar movement have gradually emerged, their function in cancer cells have remained obscure. In this study, we combine immunoprecipitation (IP), mass spectrometry (MS) and Western blot (WB) analysis to unravel their functional roles in therapy resistant lung and ovary adenocarcinoma cells by identifying their interaction partners. CABYR variants were shown to oligomerize and interact with AKAP proteins to generate a HMW signal scaffold structure, which was found to bind several glycolytic enzymes and signal transducers. Forward and reverse IP experiments followed by WB confirmed interactions between CABYR and LDH, ALDO, PFK, TPI-1, GAPDH, ENO-1 and GSK3b. Transistion from normoxic to hypoxic growth conditions disrupted the associations between glycolytic enzymes and the CABYR-AKAP signaling scaffold in the cancer cells, leading to a 3.2-fold increase in their production and secretion of lactic acid. Hypoxic growth conditions resulted in increased acetylation of lysine residues in both CTAs, and triggered deacetylation of lysines in LDH and aldolase. Treatment with resveratrol prevented hypoxia-induced dissociations, suggesting that the regulation of oxygen-sensitive protein interactions within the CABYR-AKAP-glycolysome complex involve changes in the acetylation of lysines in the engaged proteins. MS analysis of IPs finally revealed interactions between CABYR and proteins associated with the cancer cells contractile cytoskeleton. Based on these findings, it is tempting to speculate that hypoxia-induced release and subsequent local activation of glycolysomes from cytoskeleton-associated CABYR-AKAP scaffold structures might be instrumental for cancer cells ability to maintain a steady energy supply to their contractile cytoskeleton and thereby sustain their migratory and invasive capability despite encountering severe reductions in environmental oxygen levels. During the lifetime of a protein there are many points at which an acetyl group may be added to influence function. As early as during its translation, a protein may be N-terminally acetylated to preserve its stability, interactions, or subcellular localization.1 N-Terminal acetylation is a major covalent modification occurring on eukaryotic proteins, with >80% of human proteins bearing an acetyl group at the α -amino position of the first amino acid. Once a protein is properly localized, acetylation of key lysine residues can occur enzymatically or spontaneously to influence its intermolecular interactions, enzymatic functions, localization, and eventual degradation. Post-translational acetylation of lysine residues will be the primary focus of the current review. Lysine acetylation describes the transfer of an acetyl group from acetylcoenzyme A (acetyl-CoA) to the primary amine in the ε -position of the lysine side chain within a protein, a process that leads to neutralization of the position's positive electrostatic charge. Acetylation can occur nonenzymatically; however, in most known cases, the level of acetylation results from the balance of opposing enzymatic activities. Marks are "written" by lysine acetyltransferases (KATs) and "erased" by lysine deacetylases (KDACs). Acetylated lysine residues, amidst their many functions, can be functionally interpreted by a third group of proteins, the so-called "readers", which harbor specific acetyl-lysine binding domains, most prominently bromodomains. The dynamic interplay between the writers, erasers, and readers of acetylation regulates critical epigenomic and metabolic processes, in addition to other major cellular functions. Historically, investigators have focused on acetylation in the nucleus, where this mark regulates histone biology and transcription.2-5 Advances in mass spectrometric technologies have since revealed relevant targets of acetylation in nearly all intracellular compartments.6,7 Compartmentalization of cellular proteins and nutrients is essential for cell specialization and function. As such, cellular acetylation is driven by the localization of enzymes, metabolites, and cofactors required to balance acetylation and deacetylation levels. Importantly, mitochondria have emerged as organelles in which acetylation is more prominent than phosphorylation8 and plays a key role in integrating metabolic cues with the bioenergetic equilibrium of the cell. In this review, we give an overview of the chemistry and biology underlying protein lysine acetylation in mammals, review recent developments in the understanding of lysine acetylation, and provide examples of its function and regulation in distinct cellular compartments. The transfer of the acetyl group from acetyl-CoA to the ε-primary amine of a lysine residue can occur spontaneously or enzymatically. In mitochondria, acetylation is regulated in part by chemical, nonenzymatic mechanisms due to the high pH and high local acetyl-CoA concentrations within this compartment.9 The mechanism of nonenzymatic acetylation proceeds first via deprotonation of the lysine primary amine by naturally occurring hydroxide ions, followed by attack of the acetyl-CoA terminal carbonyl by the nucleophilic amine. A putative tetrahedral intermediate is transiently formed and decomposes into the reaction products acetyl-lysine, coenzyme A, and hydroxide.

Bottom Note: This work is partly presented at EuroSciCon conference on Protein, Proteomics and Computational Biology December 06-07, 2018 Amsterdam, Netherlands

EuroSciCon conference on Protein, Proteomics and Computational Biology December 06-07, 2018 Amsterdam, Netherlands