Post-Translational Modification of Proteins in Toxicological Research: Focus on Lysine Acylation

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(Received April 18, 2013; Revised June 3, 2013; Accepted June 7, 2013)

Toxicoproteomics integrates the proteomic knowledge into toxicology by enabling protein quantification in biofluids and tissues, thus taking toxicological research to the next level. Post-translational modification (PTM) alters the three-dimensional (3D) structure of proteins by covalently binding small molecules to them and therefore represents a major protein function diversification mechanism. Because of the crucial roles PTM plays in biological systems, the identification of novel PTMs and study of the role of PTMs are gaining much attention in proteomics research. Of the 300 known PTMs, protein acylation, including lysine formylation, acetylation, propionylation, butyrylation, malonylation, succinylation, and crotonylation, regulates the crucial functions of many eukaryotic proteins involved in cellular metabolism, cell cycle, aging, growth, angiogenesis, and cancer. Here, I reviewed recent studies regarding novel types of lysine acylation, their biological functions, and their applications in toxicoproteomics research.

**Key words**: Lysine acylation, Post-translational modification, Toxicoproteomics

INTRODUCTION

Post-translational modification (PTM) provides an option for expanding protein functionally in a cell or organism (1). PTMs involve protein backbone cleavages or the covalent binding of small molecules to protein residues in order to change the properties of proteins (2). In particular, covalent binding efficiently increases the diversity of proteins and changes 3D protein structures (1). As many as 300 protein PTMs have been described and found to possess fundamental biological roles (1,3). For examples, protein phosphorylation is the most intensively studied, and involves the attachment of phosphate moieties to serine, threonine or tyrosine residues by protein kinases (4). Reversible protein phosphorylation regulates most crucial cellular processes including the cell cycle, apoptosis, metabolism, signal transduction, proliferation and development (5-7).

As well as phosphorylation, acylations at lysine residues include formylation, acetylation, propionylation, butyrylation, malonylation, succinylation, and crotonylation, and these processes are crucial for functional regulations of many eukaryotic proteins (Fig. 1A). Lysine acetylation was first discovered as a post-translational modification of histones in 1964 (4). A role of histone acetylation is crucial chromatin remodeling for gene transcription since its discovery for the first 30 years (8). During the past 30 years, the biological roles of lysine acetylation have been developed in non-histone proteins. In particular, to identify protein acetylation involvement in complex biological process, the acetylome study has been develop to global analysis (8). In 2006, Kim et al., developed a method to study global protein acetylation using antibodies that selectively bind to acetylated lysine, and reported about 400 lysine acetylation sites in almost 200 proteins (9). The study revealed that > 20% of mitochondrial proteins are commonly acetylated, and the authors suggested the regulation of mitochondrial function and metabolism by reversible acetylation. Choudhary et al., identified over 3500 acetylation sites in about 1700 acetylated protein, and increased the size of the acetylome to near that of phosphorylation, the most dominant PTM (10).

Thus lysine acetylation has emerged as a key PTM in cellular metabolism, cell cycle, aging, growth, angiogenesis and cancer (11-16).

Several other PTMs, such as, propionylation, butyrylation, malonylation, succinylation, and crotonylation, at lysine residues have been discovered in the past few years (17-19). The biological functions of these novel PTMs are uncertain, and much work is being done to identify their roles in...
This review introduces biological roles of lysine acylation in toxic response and discusses recent advances in this active topic in the field of toxicology.

**Lysine acetylation.** The positively charged lysine residue plays an important role in protein folding and function. Neutralization of the charge often has a profound impact on substrate proteins. Lysine acetylation is an abundant, reversible, and highly regulated post-translational modification, which plays important roles in diverse cellular processes, such as, apoptosis, metabolism, transcription, and stress response (9). Lysine acetylation is known to be controlled by two opposing types of enzymes, acetyltransferases and deacetylase (11) (Fig. 1B). In case of fasting, toxicants exposure, and infections, the disruption of balance between two enzymatic reactions may trigger the potent toxic reaction (20). For historical reasons, the protein lysine acetyltransferases are called histone acetyltransferases (HATs), and protein lysine deacetylases is consist of histone deacetylases (HDACs) and sirtuins (21). There are three major groups of HATs: Gcn5-related N-acetyltransferases (GNATs), E1A-associated protein of 300 kDa (p300)/CREB-binding protein (CBP), and MYST proteins (22). Known HDACs are divided into Rpd3/Hda1 and sirtuin families. In humans, the former can be divided into three classes as follows: HDAC 1-3, and 8 (class I); HDAC 4-7, and 9-10 (class II); and HDAC 11 (class IV) (21). The mammalian sirtuin family comprises seven proteins (SIRT 1-7) (14). Sirtuins target a wide range of cellular proteins in nuclei, cytoplasm, and mitochondria for post-translational modification by acetylation (SIRT 1, 2, 3, and 5) or ADP-ribosylation (SIRT 4 and 6).

**Alcohol-induced protein hyperacetylation:** Recent studies have indicated that ethanol exposure induces global protein hyperacetylation (23). Mitochondrial protein hyperacetylation is a known consequence of sustained ethanol consumption and has been proposed to play a role in the pathogenesis of alcoholic liver disease (24). The mechanism is underlying acetylome alterations in lipid and fatty acid metabolism, antioxidant response, amino acid biosynthesis, and in the electron transport chain pathways. Chronic ethanol consumption substantially down-regulated hepatic SIRT 1 in mice, and was associated with an increase in the acetylated active nuclear form of sterol regulatory element-binding protein 1 in the livers from ethanol fed mice (25). Thus, alcohol consumption changes hepatic lipid metabolism and originates the development of alcoholic fatty liver.

**Exacerbated drug-toxicity by protein acetylation:** Lysine acetylation contributes to drug-induced hepatotoxicity. In mitochondria, SIRT 3 is the primary mitochondrial deacetylase that modulates mitochondrial metabolic and oxidative stress regulatory pathways (26). Mitochondrial aldehyde dehydrogenase 2 (ALDH 2) is a direct SIRT 3 substrate with an acetylation site at Lys377. The acetaminophen reactive metabolite, NAPQI, binds to ALDH 2 at Lys377 and reduces its activity (20) (Fig. 2). In other words,
Histones are formylated at multiple lysine residues located of histones and other nuclear proteins (33). Core and linker formylation generated by the metabolism of trichloroethylene (TCE) by exposure to toxicants. The major reactive compound in the formation of stable lysine adducts in proteins follow-function, which could lead to cellular deregulation and disease. Interference with epigenetic mechanisms governing chromatin formylation is relatively abundant, suggesting that it may roles in DNA binding. Moreover, in chromosomal proteins, both in the tails and globular domains of histones, in which monocytic differentiation, indicating that this modification is dynamically regulated. Furthermore, charge neutralization and nonpolar enhancement by propionylation of lysine residues appears to be highly effective for promoting surface hydrophobicity, and important driver of protein aggregation (30).

**Lysine propionylation and butyrylation.** In 2007, lysine propionylation and butyrylation were discovered in histones and confirmed by in vitro labeling and by mass spectrometry based peptide mapping (17,37). Propionyl-CoA and butyryl-CoA, are structurally similar to acetyl-CoA and differ by one or two CH₂ units. In addition, lysine propionylation has been identified in non-histone proteins in eukaryotic cells, such as, in p53, p300, and CREB binding-protein (38). Furthermore, propionylation at H3 lysine Lys23 was detected in the leukemia cell line U937 by mass spectrometry and Western analysis using a specific antibody (39). Propionylation at H3 lysine Lys23 was detected in the leukemia cell line U937 by mass spectrometry and Western analysis using a specific antibody (39). Propionylation levels in U937 cells reduce remarkably during monocytic differentiation, indicating that this modification is dynamically regulated. Furthermore, charge neutralization and nonpolar enhancement by propionylation of lysine residues appears to be highly effective for promoting surface hydrophobicity, and important driver of protein aggregation (40).

**Lysine malonylation and succinylation.** Lysine malonylation and succinylation were novel types of lysine PTMs, and were originally detected by mass spectrometry and protein sequence-database searching in 2011 (41). Lysine malonylation is a dynamic and evolutionarily conserved PTM observed in mammalian and bacterial cells, and SIRT 5, a member of the class III lysine deacetylases, can catalyze lysine demalonylation and lysine desuccinylation both in vitro and in vivo. Lysine succinylated and malonylated peptides in histone protein were verified by the MS/MS of synthetic peptides, HPLC co-elution, and isotopic labeling in HeLa cells, mouse embryonic fibroblasts, Droso-phila S2, and Saccharomyces cerevisiae cells (42). Mutagenesis of succinylation sites followed by functional assays suggested that histone lysine succinylation could have unique functional consequences.

SIRT 5 exhibits demalonylase and desuccinylase activity, in addition, lysine succinylation and malonylation are abun-
Succinyl-CoA is an intermediate in the TCA cycle and a precursor of porphyrin synthesis, whereas malonyl-CoA is the precursor of de novo fatty acid synthesis and a critical inhibitor of fatty acid oxidation. Malonyl-CoA also regulates, directly or indirectly, physiological or pathological conditions, such as, muscle contraction, cardiac ischemia, β-cell insulin secretion, and the hypothalamic control of appetite (44). Although the biological roles of lysine malonylation and succinylation are known to be related to cellular metabolism, no toxicological research has been conducted on biological roles.

Lysine crotonylation. Recently, histone lysine crotonylation was found to mark X/Y-linked genes that are active in post-meiotic male germ cells (45). The unique structure and genomic localization of histone crotonylation at lysine residues suggests that it is mechanistically and functionally different from histone lysine acetylation (46). Specifically, in human somatic and mouse male germ cell genomes, histone lysine crotonylation marks either active promoters or potential enhancers. In male germinal cells immediately following meiosis, lysine crotonylation is enriched on sex chromosomes and marks testis-specific genes, including a significant proportion of X-linked genes that escape sex chromosome inactivation in haploid cells.

### PERSPECTIVES

The emerging field of toxicoproteomics has been boosted by quantitative and qualitative proteomic technologies and their increasing applications in toxicology research (47). Toxicoproteomics uses the discovery potential of proteomics in toxicology research by applying global protein measurement technologies to biofluids and tissues after host exposure to injurious agents (48). During the past decade, toxicoproteomics has developed in parallel with proteomic technology in the following regards: the analysis of global protein expression, the use of high performance LC-MS/MS platforms and stable isotope labeling technology, targeted quantitative analysis in multiple-reaction monitoring mode, and PTM analysis by enrichment technology.

Specially, the exploration of novel PTMs and the identifications of their biological roles are a focus in the proteomic research field. Reversible PTMs can regulate the activities and localizations of intracellular proteins, and are crucial for understanding biological roles (1,3,49). Lysine acetylation, the best-known type of lysine acylation PTM, regulates various crucial roles in biological systems (9). In current review, we consider toxicity to originate from the abnormal regulation of lysine acetylation, such as, the dysregulation of modulating enzymes like acetyltransferase/deacetylase, the blocking of protein acetylation at specific lysine residues and the subsequent modulation of protein functions, and the dysregulation of histone acetylation as an epigenetic marker (24,26,30). However, new types of lysine acylations, such as, propionylation, butyrylation, malonylation, succinylation, and crotonylation continue to be detected, and their biological roles have yet to be determined.

In conclusion, the abnormal regulations of PTMs could be closely associated with the toxicities of many xenobiotics. However, although protein acylation regulates many pivotal biological functions, the possible roles of protein acylation at lysine residues in the mechanisms responsible for the toxic activities of most xenobiotics have not been comprehensively investigated. Those in the field of toxicology should pay attention to the toxicoproteomic approach involving PTMs, because the toxicology is changing, and the new paradigms arising from toxicoproteomics are des-

### Table 1. Summarizing enzymes involved in protein lysine acylation and deacylation

| Acylations | Acyltransferases | Deacylases |
|------------|-----------------|------------|
|            | Class          | Subclass   | Members       | Class          | Members       |
| Acetylation| Class I        | GNAT-family| GCN5L, PCAF   | HDAC 1,2,3,8   |
|           | A              | MYST-family| Tip60, HBOI,  | HDAC 4,5,6,7,9,10 |
|           | B              | others     | p300/CBP, TFIIC complex, etc | SIRT 1-7 |
| Formylation| unknown        |           | unknown       | unknown        |
| Propionylation| p300, CBP   |           | unknown       | SIRT 5        |
| Butyrylation| p300, CBP     |           | unknown       | SIRT 5        |
| Malonylation| unknown        |           | unknown       | SIRT 5        |
| Succinylation| unknown      |           | unknown       | SIRT 5        |
| Crotonylation| unknown      |           | unknown       | SIRT 5        |
tined to play important roles.

ACKNOWLEDGMENTS

This study was supported by a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (Grant No: A112026).

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