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HDAC1 regulates the stability of glutamate carboxypeptidase II protein by modulating acetylation status of lysine 479 residue

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ABSTRACT

Our previous study showed that the level of glutamate carboxypeptidase II (GCPII) protein is regulated by valproic acid, a histone deacetylase (HDAC) inhibitor, through acetylation of lysine residue in the GCPII protein in human astrocytes, U-87MG. The present study further investigated which HDAC subtype is involved in the acetylation of GCPII. The results revealed that GCPII interacted with HDAC1 but not with HDAC2, HDAC3, HDAC4, HDAC5, and HDAC6. Overexpression of catalytic domain (1–56 aa)-deleted HDAC1, which poorly binds to GCPII, enhanced lysine acetylation in GCPII and increased the level of GCPII protein when compared with that of the wild-type HDAC1. Further experiments showed that HDAC1 regulated the stability of GCPII protein. These data suggest that acetylation of GCPII is facilitated by HDAC1, and the acetylated GCPII is more stable than the non-acetylated GCPII. Additional experiments using siRNA HDAC1 and by *HDAC1* overexpression confirmed the role of HDAC1 in regulating the stability of GCPII protein. Further, database search of acetylation and ubiquitination sites showed four candidate lysine sites in human GCPII protein that can be both acetylated and ubiquitinylated (K207, K479, K491, and K699). Mutation (lysine residues to arginine (R)) analysis showed that in the presence of cycloheximide K479R- and K491R-hGCPII mutants were less ubiquitinylated and degraded, and decrease in the level of GCPII protein by HDAC1 was significantly blocked by K479R mutants. These data suggest that K479 is a possible site of acetylation or ubiquitination. Furthermore, the results also demonstrate that the stability of GCPII protein is regulated by HDAC1 through acetylation at the lysine 479 residue.

1. Introduction

Glutamate carboxypeptidase II (GCPII), a transmembrane zinc metallopeptidase, is found in prostate gland, intestine, and central nervous system. In the brain, GCPII is mainly expressed in astrocytic glial cells [1] and weakly in neurons. Glutamate carboxypeptidase II cleaves the neurotransmitter, N-acetyl-L-aspartyl-L-glutamate (NAAG) into N-acetyl-L-aspartate (NAA) and glutamate [1,2]. The role of NAAG is not clear. However, glutamate—an excitatory neurotransmitter—plays an important role in learning and memory, although excessive production of glutamate might cause excitotoxicity in neuronal cells, leading to neurodegenerative diseases such as multiple sclerosis, experimental autoimmune encephalomyelitis, and ischemia [3–5]. Therefore, regulation of GCPII activity and/or expression is likely to be an important in maintaining neuronal functions and pathogenesis of brain diseases.

Histone deacetylases (HDACs) are a family of enzymes that remove an acetyl group from the lysine residue of histone or non-histone proteins, thus decreasing the acetylation at lysine residue of the protein. To date, HDACs have been classified into four classes—, class I HDACs (1, 2, 3 and 8), class II HDACs (4, 5, 6, 7, 9, and 10), class III (SIRTs family) and class IV (11) [6]. Histone acetylation contributes to generate a loose chromatin configuration through a decreased ionic interaction between positively charged

Abbreviations: **GCPII**, glutamate carboxypeptidase II; **HDAC**, histone deacetylase.

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lysines of histone and negatively charged DNA, which allow a bind of transcription factor to DNA sequence. Thus, HDAC inhibition generally leads to hyperacetylation, and consequently induces gene transcription.

The recent acetylomic studies have revealed that thousands of proteins are acetylated in various cellular compartments to control a diverse biological processes [7,8]. Furthermore, these studies have also revealed that the acetylation status of protein affects transcription factor function, protein-protein interaction, and protein stability. Our previous study showed that the GCPII protein stability is enhanced by a HDAC inhibitor, valproic acid (VPA) through acetylation of lysine residue in the GCPII protein [9]. The present study aimed to (1) examine which HDAC subtype is involved in deacetylation of GCPII, (2) elucidate HDAC1 as a key deacetylating enzyme of GCPII, and (3) demonstrate that acetylation of GCPII increases the susceptibility of the protein to degradation, and acetylation at 479 lysine residue in the GCPII protein plays an important role in protein stability.

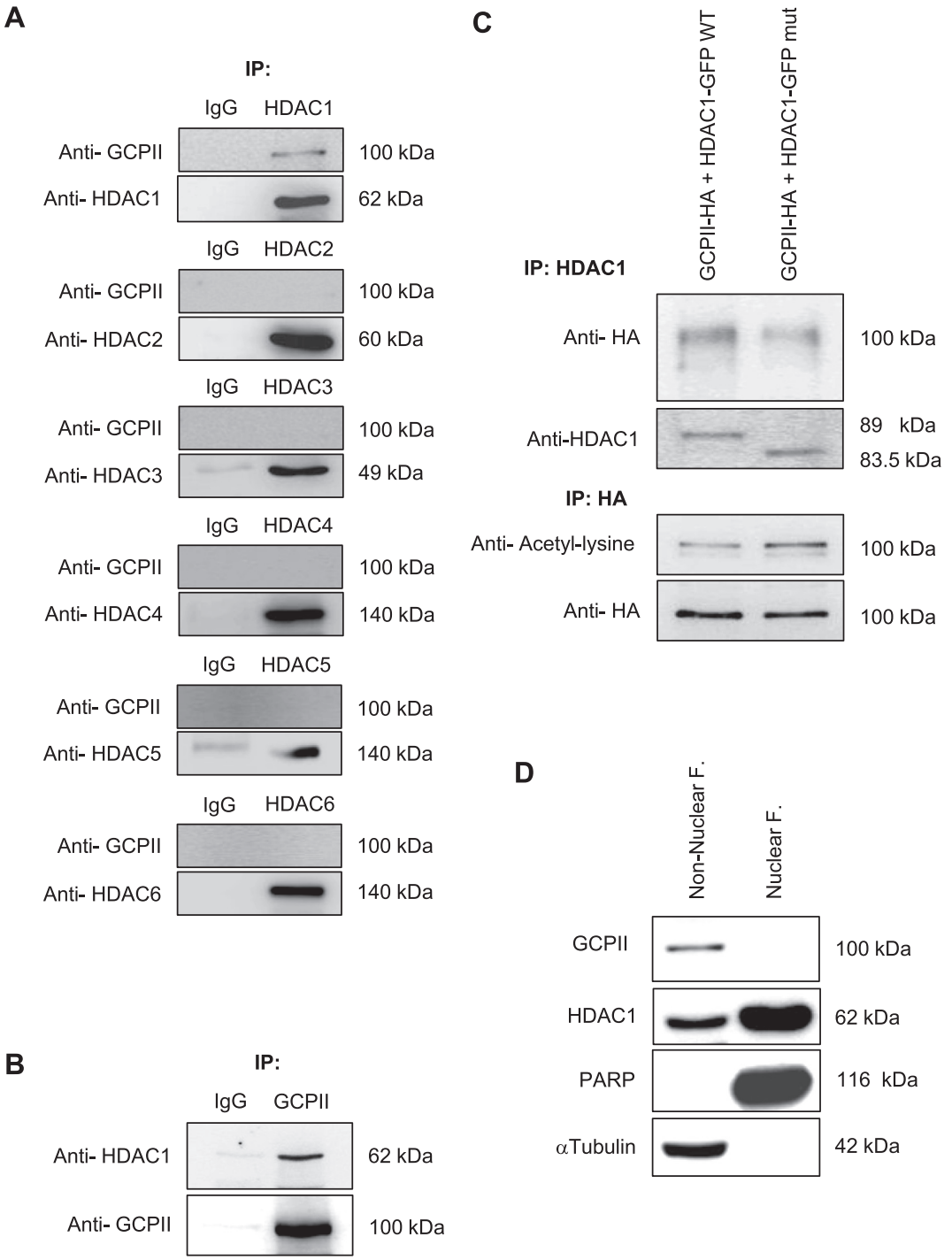

Fig. 1. GCPII binds to HDAC1. A) The band of GCPII was detected in the cell lysate pulled down with anti-HDAC1–6, respectively. B) The band of HDAC1 was observed in the cell lysate pulled down with anti-GCPII. C) The cells were cotransfected with GCPII-HA (5 μg) and HDAC1-GFP-WT (2 μg) or HDAC1-GFP mutant (2 μg) for 24 h. D) Nuclear and non-nuclear proteins were isolated.

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