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Protein Succinylation, Hepatic Metabolism and Liver Diseases

Succinylation with hepatic metabolism and diseases

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Abstract

Succinylation is one of highly conserved post-translational modifications that is processed by enzymatic and non-enzymatic manners. Succinylation exhibits strong effects on protein stability, enzyme activity, and transcriptional regulation. Protein succinylation is extensively present in the liver. Increasing evidence has demonstrated that succinylation is closely related to hepatic metabolism, such as histone acetyltransferase 1 (HAT1) promotes liver glycolysis, and the sirtuin 5 (SIRT5)-induced desuccinylation is involved in the regulation of the hepatic urea cycle and lipid metabolism. Therefore, the effects of succinylation on hepatic glucose, amino acid and lipid metabolism under the action of enzymes will be discussed here. In addition, how the succinylases regulate the processes of different liver diseases will be reviewed, such as the desuccinylation activity of sirtuin 7 (SIRT7) is closely associated with fatty liver disease and hepatitis, lysine acetyltransferase 2A (KAT2A) and HAT1 act as succinyltransferases to regulate the succinylation of target genes and influence the development of hepatocellular carcinoma (HCC). In view of the diversity and significance of protein succinylation, targeting of the succinylation pathway may serve as an attractive direction in the treatment of liver diseases.

Key Words: Protein succinylation; Hepatic metabolism; Fatty liver; Hepatitis; Hepatocellular carcinoma.

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Core Tip: Succinylation is the process of transferring succinyl groups through enzymatic and non-enzymatic means using succinyl CoA as a direct substrate. The succinylation degree could be promoted by succinyltransferases, such as lysine acetyltransferase 2A (KAT2A), histone acetyltransferase 1 (HAT1), α-ketoglutarate dehydrogenase complex (α-KGDHC), and carnitine palmitoyltransferase 1A (CPT1A). Meanwhile, desuccinylases including CobB, sirtuin 5 (SIRT5), and sirtuin 7 (SIRT7) negatively regulate the extent of protein succinylation. Several proteins and enzymes in glucose, amino acid, and lipid metabolisms are succinylated in the liver. Succinylation is also associated with the progression of several liver diseases. Thus, the proteins with varied levels of succinylation may be potential targets for the treatment of fatty liver, hepatitis, and hepatocellular carcinoma (HCC).

INTRODUCTION

1. The introduction of protein succinylation

Post-translational modification is an important way to affect protein function, integrating metabolism with physiological and pathological processes. Succinylation is an important post-translational modification of proteins *via* both enzymatic and non-enzymatic manners[1].

1.1 The process of succinylation modification

Succinylation modification is the process by which a succinyl donor transfers a negatively charged four-carbon succinyl group to the amine of lysine residues by enzymatic or non-enzymatic means[2-3] (Figure 1). The succinyl group binding to the lysine residues has a relative larger molecular weight (approximately 100.02 Da), which

significantly changes the protein structure. Additionally, the charge carried by lysine residues changes from +1 to -1, resulting in alterations in the physical and chemical properties and functions of proteins[1-4]. Succinylation modification is widespread in both cytoplasm and nucleus[5]. In the cytoplasm, succinylation is highly concentrated in mitochondria and may be involved in regulating the tricarboxylic acid cycle, amino acid metabolism, and fatty acid metabolism[6-9]. In the nucleus, lysine succinylation is present in more than 1/3 of nucleosomes, and the succinylation sites are mainly enriched in the gene promoter region, suggesting that succinylation modification may be involved in transcriptional regulation of genes[6,8-9]. Succinylated lysine residues have greater structural changes and charge differences than other typical covalent lysine modification groups such as acetyl and dimethyl[1,5]. Therefore, the influence and mechanism of succinylation on the target proteins and its potential application for the treatment of metabolic diseases have been receiving more and more attention.

1.2 Mechanisms for succinylation

On one hand, succinylation could be processed *via* non-enzymatic manners, which relies on succinyl-CoA or succinate from mitochondrial and peroxisome sources[4-5, 10-14]. Succinylation modification would occur if providing with sufficient succinyl-CoA[10]. It has been established that mixing succinyl-CoA with albumin or isocitrate dehydrogenase (IDH) increases succinylation and mitochondrial pH in pH- and dose-dependent manners[4-5]. Sreedhar *et al*[11] showed that nicotinamide adenine dinucleotide phosphate (NADPH)-specific IDH mutation results in a 280% increase of cellular succinyl-CoA levels and mitochondrial hyper-succinylation. Succinate dehydrogenase (SDH) inactivation induces excessive succinylation *via* increasing the accumulation of succinyl-CoA[12]. Notably, tissues with high levels of succinyl-CoA also show strong extent of succinylation modification, such as in the heart and in the liver[13]. Succinate entering into the cells could be converted to succinyl-CoA and enhance lysine succinylation[1]. A study has shown that dietary succinate increases succinylation of intestinal and hepatic proteins with a molecular weight of 25~35 kD in zebrafish[14].

On the other hand, the extent of succinylation could be positively regulated by several enzymes that playing succinyl-writer roles (Figure 1)[15-19], even though none specific succinyltransferase has been identified till now. For example, KAT2A is found to be also a succinyltransferase[15-16], which has been reported to upregulate H3K79 succinylation and β-catenin stabilization, thereby promoting glycolysis[20]. Zhou *et al*[21] confirmed that KAT2A promotes the succinylation of K46 and K280 of C-terminal binding protein 1 (CTBP1) and mediates the transcription suppressing activity. In addition, HAT1 is identified as a succinyltransferase of both histone and non-histone proteins[17, 22]. HAT1 mediates the succinylation of histones, and quantitative proteomic analysis revealed five succinylation sites on 45 histones[17]. One research has shown that HAT1 is necessary for the regulation of epigenetic and gene expression by H3K122 succinylation[17]. Wang *et al*[22] and Yang *et al*[17] demonstrated that phosphoglycerate mutase 1 (PGAM1), a critical enzyme in glycolysis, is succinylated by HAT1 at K99. The later report also mentioned that aspirin down-regulates HAT1 by targeting NF-κB to induce PGAM1 K99 de-succinylation to suppresses the glycolysis process[22]. Furthermore, the α-ketoglutarate dehydrogenase complex (α-KGDHC) regulates succinylation either by regulating succinyl-CoA levels or by directly catalyzing succinylation[4,18]. Inhibition of α-KGDHC reduces succinylation levels of both cytoplasmic matrix and mitochondrial proteins[4]. The E2k subunit of α-KGDHC is demonstrated to be essential for its trans-succinylase activity. The absence of E2k subunit reduces succinylation, while the presence of alpha-ketoglutaric acid increases succinylation[4]. Another lysine succinyltransferase in mammalian cells is carnitine palmitoyltransferase 1A (CPT1A)[19]. Kurmi *et al*[19] evidenced that CPT1A can play a role of succinyltransferase both *in vivo* and *in vitro* to regulate substrate proteins and related metabolic processes. Wang *et al*[23] discovered that CPT1A-mediated succinylation of S100A10 (a protein that is overexpressed in gastric cancer) increases human gastric cancer invasion. Moreover, CPT1A promotes the succinylation of mitochondrial fission factor (MFF) at K302 and enhances the development of ovarian cancer[24].

Thirdly, significant progress has been made in the exploration of desuccinylases that negatively regulate succinylation (Figure 1). CobB is the first desuccinylase discovered in prokaryotes with both deacetylation and desuccinylation activities[25]. The HPLC assay showed that CobB could deacetylate and desuccinylate a histone H3K9 peptide with similar efficiency, whereas the desuccinylation activity of CobB might be induced when cells are treated with succinate[25]. SIRT5 and SIRT7 are currently known important desuccinylases in eukaryotes[26-32]. SIRT5 acts in all cell compartments. The activity of SIRT5 is dependent on NAD^+, which is influenced by the availability of NAD^+ (substrate) and the amount of nicotinamide (product)[26]. In SIRT5 KOs, more than 80% of proteins are succinacylated in the TCA cycle to enhance cell respiration, and 60% of proteins in fatty acid metabolism are succinylated[27]. At least 2,565 succinylation sites on 779 proteins in mammalian fibroblasts and liver tissues were found to be regulated by SIRT5[27]. Novel targets for SIRT5 in regulating the mitochondrial lysine succinylome such as uncoupling protein 1 (UCP1) in mouse brown adipose tissue was recently notified[28-29]. SIRT7 is a member of sirtuin family proteins that are described as NAD(+)-dependent class III histone deacetylases[30-31]. A research indicated that SIRT7 catalyses the desuccinylation of H3K122 which promotes chromatin condensation and DNA double-strand break repair[30]. Yu *et al*[31] showed that SIRT7 restricts chronic hepatitis B virus (HBV) transcription and replication through catalyzing desuccinylation of histone H3 (H3K122) that associated with cccDNA minichromosome. SIRT7 mediates the desuccinylation of arginine methyltransferase 5 (PRMT5) K387, which is involved in lipid reprogramming, tumor growth and metastasis[32].

Up to now, characterization of succinyltransferases and desuccinylases, the target specificity, the function of succinylation, and its clinical application still need to be further investigated, which is also of significance for proteomic analysis.

Figure 1 Mechanisms for succinylation.

Succinylation is the process of transferring a negatively charged four-carbon succinyl groups to amines of lysine residues through enzymatic and non-enzymatic manners using succinyl-CoA as a direct substrate. The succinylation degree could be promoted by succinyltransferases, such as KAT2A, HAT1, α-KGDHC, and CPTIA. Meanwhile, desuccinylases, including CobB, SIRT5, and SIRT7 negatively regulate the extent of protein succinylation.

2. The effects of succinylation on hepatic metabolism

The liver is one of crucial metabolic organs, in which the main metabolic processes including glucose, amino acid, and lipid metabolisms occurring[33]. The overall abundance of lysine succinylation in liver is higher than in other tissues, with proteins and enzymes in several metabolic pathways being succinylated [34].

2.1 The influence of protein succinylation on glucose metabolism and amino acid metabolism

Glucose homeostasis is vital for health, which is largely regulated by hepatic glycogen synthesis, gluconeogenesis, and glycolysis[35-36]. Enhancement of glycolysis is a contributor for the growth of tumor cells. Yang *et al*[21] performed KEGG pathway enrichment analysis on HAT1-targeted non-histone proteins and found that HAT1 mediates the succinylation of glycolytic related proteins, including seven key enzymes such as GPI, TPI, GAPDH, PGK, PGAM, Enolase, and PKM. The authors further demonstrated that the HAT1-induced K99 succinylation of PGAM1 increases its activity, which further promotes tumourgenesis[21]. Wang *et al*[20] showed that aspirin reduces HAT1 expression, which decreases the K99 succinylation level of PGAM1, thereby restricting the PGAM1 activities and inhibiting glycolysis in liver cancer (Figure 2).

The liver is also a major tissue for the conversion of ammonia[37], which is a toxic metabolite in amino acid metabolism under physiological conditions[38]. For the conversion of ammonia to non-toxic urea *via* urea cycle, carbamoyl phosphate synthase 1 (CPS1) is the first enzyme that highly abundant in mitochondria, which is expressed mainly in hepatocytes[39]. Polletta *et al*[40] demonstrated that mitochondrial SIRT5 not

only promotes ammonia detoxification by catalyzing desuccinylation of CPS1 , but also regulates glutamine homeostasis and ammonia levels by inhibiting glutaminase (GLS) activity to reduce ammonia release and conversion of glutamine to glutamate (Figure 2). Additionally, Zhang *et al*[41] conducted stoiometry of lysine succinylation in mouse liver, and found several highly succinylated lysine sites in arginine succinate synthetase (ASS1), a key enzyme in the urea cycle, which were regulated by SIRT5. Metabolomic analysis has confirmed that SIRT5 deficiency reduces liver urea cycle activity, and more importantly, SIRT5 deficiency affects ammonia tolerance.

2.2 The influence of protein succinylation on lipid metabolism

The liver serves as an important regulator of lipid homeostasis[42], which includes lipid uptake, lipogenesis, fatty acid oxidation, ketogenesis, and lipid secretion[43]. When lipid synthesis exceeds lipolysis or export, it causes the accumulation of lipids in hepatocytes, ultimately leading to hepatic steatosis[32,44]. PRMT5 is a type II arginine methyltransferase that affects a variety of metabolites including phospholipids, fatty acids, and steroid hormones. Yuan *et al*[32] demonstrated that SIRT7-mediated desuccinylation of PRMT5 at K387 increases its methyltransferase activity, thereby up-regulating lipid metabolism-related factors, such as SREBP1a, FASN, ACACA, PPARγ, SCD, *etc.* Moreover, SIRT5 is also involved in the regulation of fatty acid β-oxidation[52]. When SIRT5 is deficient, fatty acid β-oxidation was reduced, which leads to fat accumulation in the liver[13].

Ketone bodies are produced by the liver in the deficiency of glucose through fatty acid catabolism[45-46], which are composed of acetoacetic acid (AcAc), β-hydroxybutyrate (BHB), and acetone[47]. Mitochondrial 3-hydroxy-3- methylglutaryl-CoA synthetase 2 (HMGCS2) is a key enzyme required for ketogenic biosynthesis, which is regulated by succinylation[48]. Early studies on ketogenic regulation have shown that accumulation of succinyl-CoA is the main process leading to enzyme inactivation in the liver. It was reported that glucagon drastically reduces succinyl-CoA levels and HMGCS2 succinylation, which leads to strong ketogenic activation[4]. SIRT5 induces desuccinylation of HMGCS2 and promotes ketone body formation (Figure 2). Among

the 15 succinylated lysine residues identified on HMGCS2, several sites appear to be highly targeted by SIRT5 including K83, K310, K350, K354 and K358[49]. Studies have shown that lysine adjacent to the HMGCS2 substrate binding site is strongly succinylated, suggesting that succinyl-CoA may interact with lysine residues around the catalytic pocket, resulting in non-enzymatic modification of these lysines[50-51].

At present, the enzymes in liver glucose, amino acid and lipid metabolism regulated by succinylation are only the tip of the iceberg, and whether other enzymes in liver are modulated by succinylation remain to be further explored.

Figure 2 The effect of succinylation on hepatic metabolic pathways.

The influence of succinylation on hepatic glucose metabolism: under the stimulation of succinyl-CoA, HAT1 causes the K99 site of PGAM1 to be succinylated and promotes its enzyme activity, thus promoting glycolysis. The influence of succinylation on hepatic amino acid metabolism: SIRT5 promotes urea production by regulating the desuccinylation of ASS1 and CPS1. The influence of succinylation on hepatic lipid metabolism: SIRT5 induces desuccinylation of HMGCS2 and promotes ketone body formation.

3. The relationship of succinylation with liver diseases

Large quantity of evidences have established that succinylation is strongly associated with the progression of liver diseases, primarily for fatty liver, hepatitis, and HCC. Succinylation not only regulates fat deposition and thus fatty liver degeneration[52-53], but also promotes HBV transcription and replication[21]. In addition, succinylation stimulates immune escape and tumor growth in HCC[54]. Therefore, the specific roles of succinylation in liver diseases are discussed herein.

3.1 Succinylation is involved in fatty liver disease

Fatty liver is one of common chronic diseases with a high prevalence worldwide, which is caused initially by excessive fat accumulation in the liver[55-56]. As one of metabolism-related post-translational modifications, the succinylation degree is enhanced in fatty liver samples[52,57]. Cheng *et al*[57] conducted quantitative succinylated proteome analysis

in the liver of NAFLD rat models, and identified 178 differentially succinylated proteins, which are involved in various metabolic and cellular processes, and promote the progression of NAFLD to varying degrees. Another study[52] also indicated that overexpression of SIRT5 in the liver results in decreased succinylation, enhanced fatty acid oxidation, and attenuated fatty liver degeneration. Sterol-regulatory element binding protein 1 (SREBP1), one of the transcription factors regulating hepatocellular lipogenesis, induces the expression of several lipogenic genes[58]. Guo *et al*[53] found that histone deacetylase 1 (HDAC1) stabilized by P50 maintains SREBP1c activity through desuccinylation and promotes hepatic steatosis (Figure 3A). Yuan *et al*[32] verified that SIRT7-mediated desuccinylation of PRMT5 at K387 promotes fatty liver by inducing arginine methylation of SREBP1a (Figure 3A). In summary, the proteins with varied levels of succinylation may be potential targets for the treatment of fatty liver.

3.2 Succinylation promotes hepatitis virus replication

Viral hepatitis is an infectious disease threatening human health with a rising incidence in recent years[59]. HBV is a hepatotropic DNA virus that encodes multiple gene products for viral replication[60-62]. cccDNA plays an important role as a template for HBV transcription[63]. In the nucleus of HBV-infected cells, SIRT7 catalyzes the desuccinylation of cccDNA-bound histone H3K122, thereby limiting HBV transcription and replication[31]. KAT2A is identified as an important host factor for HBV replication[16]. Wang *et al*[15] confirmed that KAT2A is coupled to nuclear α-KGDHC, which acts as a histone H3 succinyltransferase. A later research[64] found that KAT2A can bind to cccDNA by interacting with the HBV core protein and catalyzing the succinylation of H3K79 on cccDNA (Figure 3B), thus promoting cccDNA transcription. Interestingly, Yuan *et al*[65] discovered that IFN-α restrains HBV cccDNA by down-regulating KAT2A-mediated histone H3K79 succinylation. Collectively, targeting succinyl-modification enzymes and the succinylated proteins may provide new perspectives for the treatment of HBV.

3.3 Succinylation degree is associated with the progression of HCC

HCC is a common and highly lethal cancer, which is affected by many factors and ranks the fourth in cancer incidence and the second in cancer mortality[66-67]. In liver cancer patients, the expression of SIRT7 is significantly higher than that in normal liver tissues, which initially rises in the first and middle stages of hepatocellular carcinoma, but tends to decrease in the later stages[68]. Moreover, the deficiency of SIRT5 promotes HCC and is associated with oxidative damage response[54]. Sun *et al* [54] showed that SIRT5 depletion leads to increased lysine succinylation of acyl-CoA oxidase 2 (ACOX2) (Figure 3C), resulting in the synthesis of primary bile acids, which further promotes immune escape and tumor growth in HCC. In addition, Yang *et al*[21] confirmed that HAT1 promotes cell proliferation in HCC by catalyzing H3K122 succinylation (Figure 3C). Aspirin inhibits the succinylation level of PGAM1 at K99 by down-regulating the expression of HAT1, decreases the level of glucose consumption and lactic acid production of liver cancer cells, thereby attenuating the glycolytic pathway in HCC[20,69]. In view of the complex roles of the succinylation signaling pathway in HCC, further studies are necessary in order to distinguish the pleiotropic effects of succinylation for its application in treating liver cancers.

Figure 3 Succinylation affects the progression of fatty liver, hepatitis, and HCC.

A: P50 stabilizes HDAC1 protein to keep desuccinylation of SREBP1c, thereby promoting fatty liver. SIRT7-mediated desuccinylation of PRMT5 at K387 promotes fatty liver by inducing arginine methylation of SREBP1a; B: IFN-α inhibits KAT2A-mediated succinylation of histone H3K79 and SIRT7 promotes desuccinylation of histone H3K122, both of which restrain viral replication and thus hepatitis; C: SIRT5 deficiency activates ACOX2 succinylation, leading to elevated bile acid levels and promoting HCC. HAT1 not only promotes hepatocellular carcinogenesis by activating H3K122 succinylation, but also promotes the glycolytic pathway by promoting succinylation of PGAM1 at K99 , thereby promoting HCC.

CONCLUSION

Through delineating the pleiotropic relationships between succinylation and hepatic metabolism, it can be seen that protein succinylation is involved in various physiological and pathological processes of liver. Despite the significant progress in this kind of post-translational modification, many issues remain unresolved and provide opportunities for future studies.

Succinylation is site-specific, and some proteins have several succinylation sites to make the substrate perform varied biological functions. Research has found that the 252 identified succinylated proteins have 1,190 SuK sites and a total of 6,579 Lysines, with at least 18% of lysines on these proteins being modified by succinylation [49]. Whether these lysine succinylation sites overlap with known enzyme active sites may be an important sign for the function of succinylation regulation. Therefore, further research on the exact influences and mechanisms for succinylation on different proteins and/or different lysine sites of one target protein are of great importance.

Some specific succinylases regulate glycolysis, amino acid, and lipid metabolism by modifying the succinylation degree of critical enzymes. Are there other succinylases that are crucial for hepatic metabolism? HDAC1, a histone deacetylase, maintains SREBP1c activity through desuccinylation and promotes hepatic steatosis[53]. Similarly, some succinylation modifying enzymes also exert other enzymatic activities. For instance, the demalonylation activity of SIRT5[27] and the acetylation activity of KAT2A are likely to contribute to regulate the biological processes of the liver as well. This suggests that some enzymes with other functions can also exert succinylation or desuccinylation activity, and some identified succinylases may act as other enzymatic activity to participate in metabolic reactions.

In addition, the succinylation-regulated metabolic processes could affect the progression of fatty liver, hepatitis and HCC. In some cases, the effect of succinylation on disease development may be not common between histone and non-histone proteins. For instance, Yuan *et al*[32] verified that SIRT7-mediated desuccinylation of PRMT5 at K387 promotes fatty liver. Meanwhile, SIRT7 catalyzes the desuccinylation of

cccDNA-bound histone H3K122, thereby limiting HBV transcription and replication[16]. This indicates that the roles of succinyltransferase / desuccinylase are not consistent in different metabolic environments or reactions. Therefore, we ask the following scientific questions to be resolved. What are the differences of succinylation levels and regulatory mechanisms in the occurrence and development of various metabolic diseases at different stages? How can we modulate more succinylation-related pathways in target tissues to improve human health?

In conclusion, the in-depth study of these issues would greatly enhance our understanding on protein succinylation, which further support the theoretical basis for the treatment of metabolic diseases and the development of related drugs.

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