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REVIEW ARTICLE

Protein post-translational modification by lysine succinylation: Biochemistry, biological implications, and therapeutic opportunities

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Abstract Lysine succinylation (Ksuc) is a novel protein post-translational modification (PTM) wherein a succinyl group modifies a lysine residue. Ksuc leads to significant chemical and structural changes to the modified protein. Recent studies have shown that Ksuc might play an important role in organism physiology and some pathophysiological processes, such as tumorigenesis and metabolic diseases. To provide an understanding of the molecular mechanism and functions of Ksuc in different organisms, we reviewed the current literature about Ksuc, mainly summarizing the research advances in eukaryotes and prokaryotes based on both traditional study methods and site prediction tools. We also discussed inhibitors or activators associated with Ksuc that may contribute to proteomic studies and could be useful in future clinical practice. A deeper understanding of Ksuc may shed new light on life science at the protein level and could lead to novel therapeutic strategies for various diseases.

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Introduction

Protein post-translational modification (PTM), an evolutionarily conserved strategy in organisms that enables them to rapidly respond to various physiological conditions,[1] is considered a potent biological mechanism to increase proteome diversification.[2] Currently, more than 300 kinds of PTMs have been identified to occur as part of both normal physiological processes and in numerous diseases.[3] Among the PTMs, methylation,[4] acetylation,[5] and phosphorylation[6] have been the most thoroughly studied. Increasing evidence suggests that PTMs are critically regulated in cells, and result in diverse effects on protein function and cell structure.[7]

Lysine is one of the three amino acids (lysine, arginine, and aspartic acid) that have a positively charged side chain under physiological pH, which makes it possible for lysine residue side chain to be involved in non-covalent interactions, such as electrostatic neutralizing interactions with negatively charged residues.[8] This underlies the importance of lysine in the conformational changes of proteins and protein—protein interactions.

Lysine succinylation, an evolutionarily conserved PTM, is a process wherein a succinyl donor covalently binds a succinyl group (—CO—CH_2-CH_2-CO_2H) to one or more protein lysine residues through enzymatic or non-enzymatic means.[9] Among the various amino acids, lysine residues are the most frequent target sites for succinylation.[10] Succinylation causes the conjugation of lysine residues with large molecular weight succinyl groups, resulting in significant changes in the protein structure. At physiological pH, succinylated lysine residues can induce charge mutations, and the charge carried by lysine residues is changed from +1 to −1, resulting in major changes in the physical and chemical properties of proteins, as well as their functions. Compared with methylation and acetylation, succinylation may have a greater effect on protein properties.[11,12]

Considering the importance of Ksuc in regulating the structure and function of various proteins, it is necessary to better understand the process. In this review, we highlight the emerging evidence supporting the role of Ksuc in health and disease based on biochemical (cellular function), physiological (organism models), and pathological (pathological samples) studies. We also discuss some of the research methods used to study Ksuc and some compounds that can affect Ksuc levels. This article's main content is summarized in Figure 1.

Discovery of lysine succinylation

To systematically review the roles of Ksuc in eukaryotes and prokaryotes, we searched the NCBI PubMed database for all eligible papers. Search terms and keywords include the following: "lysine succinylation" or "Ksuc" or "Ksucc". The results provided 632 papers as of November 27, 2021. Subsequently, related articles were manually curated by Guo Zhao and Longxiang Xie. The language of the related papers was confined to English. Any discrepancy about the papers inclusion and exclusion of papers was discussed among our group. Finally, 132 papers were included that met the criteria described in Figure S1.

Lysine succinylation was first reported in 2011 by Zhang et al.[12] They used mass spectrometry and protein sequence alignment for the initial identification of the succinyllysine residue. Subsequently, they applied a mass spectrometry-based proteomic method, which combined Western blot analysis, *in vivo* labeling with isotopic succinate, and HPLC-MS/MS to verify the succinyllysine peptides derived from proteins *in vivo*. Importantly, Ksuc is an evolutionarily conserved PTM, and succinyl coenzyme A (succinyl-CoA), an important metabolic intermediate, may be a cofactor for Ksuc. This suggests that Ksuc may affect multiple metabolic pathways in cells. Moreover, at physiological pH (7.4), Ksuc changes the charge state of lysine from +1 to −1, which results in a dramatic alteration in the protein structure. Considering the high abundance of Ksuc and its subsequently induced biochemical changes, this modification has been attracting increasing attention from the research community (Fig. 2).

Biochemical process of succinylation modification

Succinyl-CoA serves as the main acyl donor molecule for the Ksuc reaction,[12] while SIRT5 and SIRT7 can catalyze lysine desuccinylation *in vivo* and *in vitro*.[13;14] Recently, it has been reported that Ksuc can also be achieved enzymatically in cellular histones.[15] We provide a summary of the non-enzymatic reaction, enzymatic succinylation and enzymatic desuccinylation in Table 1.

Non-enzymatic lysine succinylation by succinyl-CoA

Previous studies have shown that Ksuc can occur via a non-enzymatic process where the succinyl group is derived directly from succinyl-CoA[16,17] (Fig. 3). Weinert et al showed that succinylation in yeast cells is dependent on succinyl-CoA provided by the TCA cycle. They performed succinylation analyses on different organisms, with succinyl-CoA as the succinyl donor of lysine at a concentration that affected succinylation levels.[18] Succinyl-CoA is mainly formed in the mitochondria by odd-numbered fatty acid oxidation or the TCA cycle. However, a previous study revealed that succinic acid can traverse the mitochondrial membrane to affect the Ksuc level in the cytoplasm.[19] Interestingly, researchers have also found GTP-dependent succinyl-CoA synthetase in the cytoplasm.[20] This implies that succinyl-CoA may be localized both inside and outside the mitochondria, and the Ksuc process determined by succinyl-CoA occurs in several different compartments within the cell. Subsequent studies found that more than 1/3 of all nucleosomes, including histone and non-histone chromatin, were succinylated, suggesting that the succinylation of chromatin may also affect gene expression in the nucleus.[21] Another study showed that only 8% of the succinylated proteins in yeast were generated in the mitochondria, and the majority of lysine succinylation occurred in the cytosol and nucleus via succinic acid or some succinic acid metabolites. The frequent occurrence of Ksuc outside of mitochondria indicates that succinyl-CoA, succinic acid or some succinic acid metabolites may also affect the Ksuc in the cytosol and nucleus.[18] Similarly,

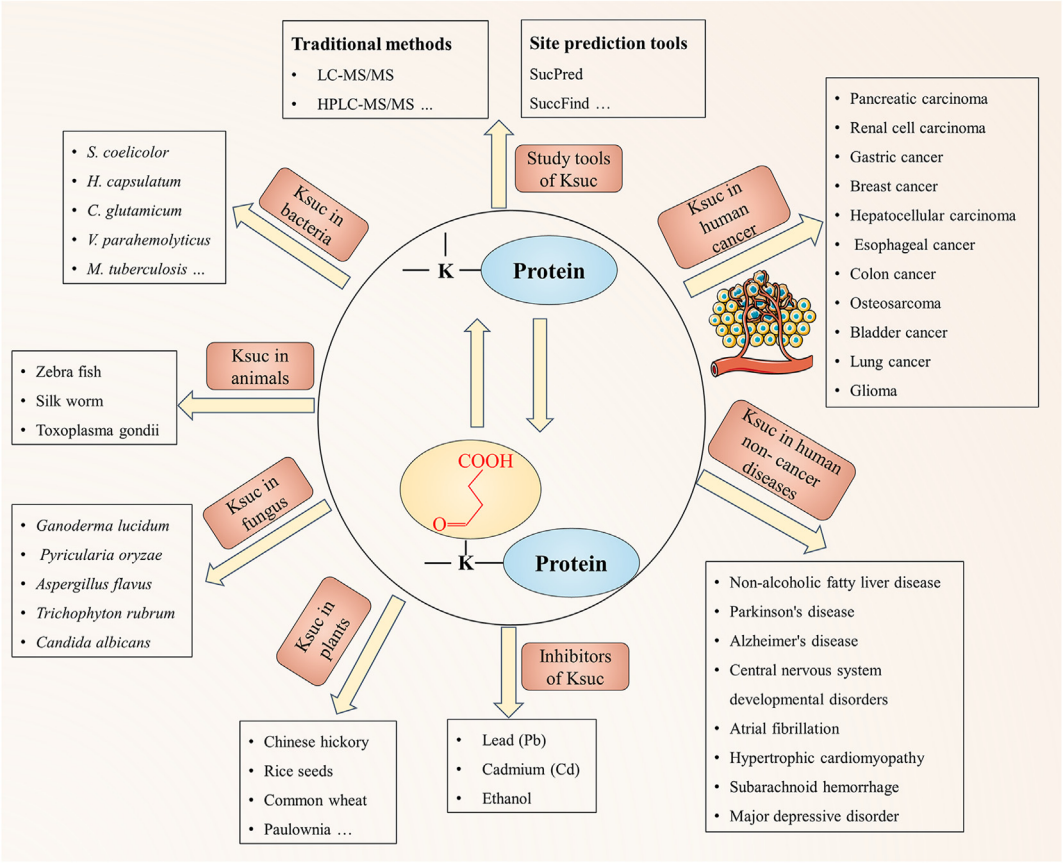

Figure 1 Schematic overview of this paper. In this review, we discussed the emerging roles of protein post-translational modification by lysine succinylation in different organisms based on normal organism models (animals, plants, fungus, and bacteria) or pathological models (human cancers and some non-cancer diseases). We also summarized the study methods on Ksuc and some compounds, including inhibitors and activators, that can affect Ksuc levels.

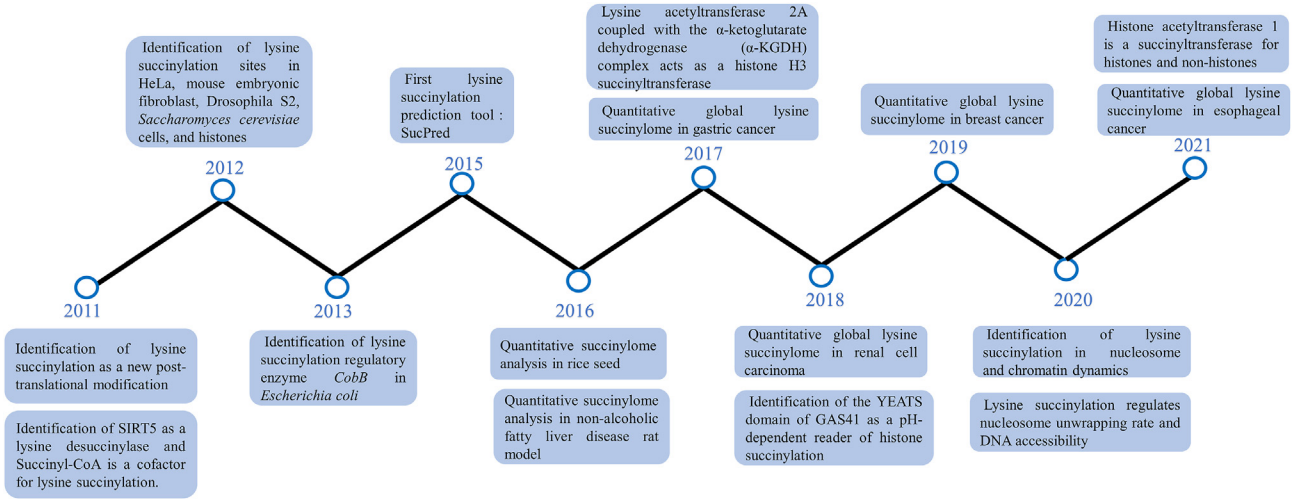

Figure 2 The discovery and development history of lysine succinylation in diverse organisms. From 2011 to 2021, numerous studies of lysine succinylation have been reported. We selected 16 representative works in this field to mark the discovery and development history of lysine succinylation in diverse organisms.

Zhang et al used isotope labeling to demonstrate that succinic acid, as an intermediate metabolite of the tricarboxylic acid cycle, can become a source of succinyl groups.[12] In brief, these studies support that succinyl-CoA, succinic acid, and/or other succinyl-metabolites may affect Ksuc both within and outside the cell mitochondria.

Table 1 The succinyltransferase, desuccinylases, non-enzymatic reaction, and reader of lysine succinylation.

Name	Amino acid length	Species	Methods	Succinyltransferase/ Desuccinylases/Non-enzymatic reaction/ Reader	Function	References
SIRT5	310	Mice	LC-MS/MS	Desuccinylases	Deletion of SIRT5 in mice appears to increase level of succinylation on carbamoyl phosphate synthase 1, which is a known target of SIRT5.	[32]
SIRT5	310	Mice	HPLC-MS/MS	Desuccinylases	SIRT5 attenuates pyruvate dehydrogenase complex (PDC) activity in the TCA cycle and suppresses SDH activity and cellular respiration	[49]
SIRT5	310	Human	NA	Desuccinylases	SIRT5 binds to, desuccinylates and activates Superoxide dismutase 1 (SOD1). SOD1-mediated ROS reduction is increased when SIRT5 is co-expressed.	[46]
CobB	279	*E. coli*	Nano-HPLC-MS/MS	Desuccinylases	CobB is a bifunctional enzyme with lysine desuccinylation and deacetylation activities	[30]
SIRT5	310	Mice	Reverse-phase LC-ESI-MS/MS	Desuccinylases	SIRT5 regulates succinylation of the rate-limiting ketogenic enzyme 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2) both *in vivo* and *in vitro*. And loss of SIRT5 leads to accumulation of medium- and long-chain acylcarnitines and decreased β-hydroxybutyrate production *in vivo*.	[13]
SIRT5	310	Mice	LC-MS/MS	Desuccinylases	SIRT5-KO exacerbates ischemia/reperfusion injury. And increased I/R injury in SIRT5−/− hearts depends on succinate dehydrogenase activity.	[87]
SIRT5	310	Mice	HPLC-MS/MS	Desuccinylases	SIRT5 regulates ECHA enzymatic activity mainly through desuccinylation of Lys351.	[36]
SIRT5	310	Human	NA	Desuccinylases	SIRT5 binds to, desuccinylates and inhibits PKM2 activity at K498. And succinylation at K498 of PKM2 sensitizes cells to oxidative damage and suppressed cell proliferation and tumor growth	[39]
SIRT5	310	Mice	Reverse-phase LC-ESI-MS/MS	Desuccinylases	SIRT5 counteracts succinylation of mitochondrial membrane proteins and is targeted to inner mitochondrial membrane proteins via affinity for cardiolipin to promote respiratory chain function.	[44]
SIRT5	310	Mice	LC-MS/MS	Desuccinylases	SIRT5 deacylated metabolism-related proteins and attenuates hepatic steatosis in ob/ob mice.	[35]
SIRT5	310	Human	LC-MS/MS	Desuccinylases	SIRT5 promotes ccRCC tumorigenesis through inhibiting SDHA succinylation.	[41]
SIRT5	310	Mice	LC-MS/MS	Desuccinylases	SIRT5 KO leads to increased lysine succinylation and decreased ATP synthase activity.	[138]
SIRT5	310	Mice	nLC-DIA-MS/MS	Desuccinylases	Brown adipose tissue (BAT) specific-SIRT5 KO elevates protein succinylation and malonylation levels. And mutation of the two succinylated lysine in uncoupling protein 1	[38]

(*continued on next page*)

Table 1 (*continued*)

Name	Amino acid length	Species	Methods	Succinyltransferase/ Desuccinylases/Non-enzymatic reaction/ Reader	Function	References
					(UCP1) to acyl-mimetic glutamine and glutamic acid significantly decreases its stability and activity.	
SIRT5	310	Human	NA	Desuccinylases	SIRT5 suppresses GC cell growth and migration through desuccinylating 2-oxoglutarate dehydrogenase (OGDH) and inhibiting OGDH complex activity to disturb mitochondrial functions and redox status.	139
Sc CobB2	241	*Streptomyces coelicolor*	HPLC-MS/MS	Desuccinylases	ScCobB2 regulates S. coelicolor protein biosynthesis and carbon metabolism pathways.	31
SIRT5	310	Mice	NA	Desuccinylases	SIRT5-catalyzed desuccinylation of mitochondrial antiviral signaling protein (MAVS) at K7 diminishes the formation of MAVS aggregation after viral infection, resulting in the inhibition of MAVS activation and leading to the impairment of type I IFN production and antiviral gene expression.	140
CobB	257	*A. hydrophila*	LC-MS/MS	Desuccinylases	cobB mediated lysine desuccinylation and deacetylation in *A. hydrophila*. And cobB promoted biofilm formation, migration activity, and was sensitive to oxidative stress.	141
SIRT7	402	Human	LC-MS/MS	Desuccinylases	SIRT7-catalysed H3K122 desuccinylation is critically implemented in DNA-damage response and cell survival.	14
SIRT5	310	Human	LC-MS/MS	Desuccinylases	SIRT5 mediates desuccinylation of p53 at K120, resulting in the suppression of p53 activation.	142
SIRT7	402	Human	Closed circular DNA (cccDNA) ChIP-Seq approach	Desuccinylases	SIRT7, as an NAD + -dependent histone desuccinylase, could bind to cccDNA through interaction with hepatitis B virus (HBV) core protein where it catalyzed histone 3 lysine 122 (H3K122) desuccinylation.	143
SIRT5	310	Human	LC-MS/MS	Desuccinylases	In human non-tumor HEK293 cell lines, human reactive intermediate deaminase A (hRIDA) K-succinylation was negatively correlated to the cell proliferation rate and was under the control of SIRT5.	144
SIRT5	310	Human	UPLC-MS/MS	Desuccinylases	SIRT5 inactivation led to the mitochondrial serine hydroxymethyltransferase SHMT2, enzymatic downregulation and to abrogated cell growth under metabolic stress.	42
Succinyl-CoA	289	*E. coli*	HPLC-MS/MS	Non-enzymatic reaction	Succinyl-CoA might be a cofactor for lysine succinylation.	12
Succinyl-CoA	427	Yeast	LC-MS/MS	Non-enzymatic reaction	Succinylation was globally altered by growth conditions and mutations that affected succinyl-coenzyme A (succinyl-CoA) metabolism in the tricarboxylic acid cycle. And lysines that are acetylated are also frequently targets of succinylation.	18

Succinyl-CoA	520	Human	SILAC-based quantitative proteomics approach	Non-enzymatic reaction	SDH loss TCA cycle defect results in succinyl-CoA increase and hypersuccinylation	[21]
Succinyl-CoA	520	Human	LC-MS/MS	Non-enzymatic reaction	Increased succinyl-CoA levels contribute to the pathology of SCL deficiency through post-translational modifications.	[145]
YEATS domain of GAS41	227	Human	NA	Reader	GAS41 YEATS domain presents significant binding affinity toward H3K122suc upon a protonated histidine residue.	[25]
α-KGDHC	453	Human	LC-MS/MS	Succinyltransferase	α-KGDH complex enables KAT2A to access the concentrated succinyl-CoA generated locally by the α-KGDH complex and thereby compensates for the low concentration of succinyl-CoA in the nucleus. And H3K79 succinylation by α-KGDH-coupled KAT2A promotes tumor growth.	[23]
KAT2A	837	Human	LC-MS/MS	Succinyltransferase	α-KGDH complex enables KAT2A to access the concentrated succinyl-CoA generated locally by the α-KGDH complex and thereby compensates for the low concentration of succinyl-CoA in the nucleus. And H3K79 succinylation by α-KGDH-coupled KAT2A promotes tumor growth.	[23]
CPT1A	773	Human	SILAC-based quantitative proteomics approach	Succinyltransferase	CPT1A lysine succinylated its substrate proteins *in vivo* and *in vitro*, and such KSTase activity inhibits enolase 1 and promotes cell proliferation under glutamine depletion independent of its classical CPTase activity.	[26]
CPT1A	773	Human	LC-MS/MS	Succinyltransferase	CPT1A-mediated succinylation of S100A10 increases human gastric cancer invasion.	[27]
KAT2A	837	Human	NA	Succinyltransferase	KAT2A-mediated H3K79 succinylation in the promoter region of YWHAZ is required for 14-3-3ζ expression and subsequent β-catenin stability.	[24]
CPT1A	773	Human	HPLC-MS/MS	Succinyltransferase	CPT1A succinylates lactate dehydrogenase A (LDHA) on K222, which thereby reduces the binding and inhibits the degradation of LDHA, as well as promotes GC invasion and proliferation.	[82]
HAT1	419	Human	LC-MS/MS	Succinyltransferase	HAT1 succinylates histone H3 on K122, contributing to epigenetic regulation and gene expression in cancer cells. Moreover, HAT1 catalyzes the succinylation of PGAM1 on K99, resulting in its increased enzymatic activity and the stimulation of glycolytic flux in cancer cells.	[80]
α-KGDHC	453	Human	Nano-MS/MS	Succinyltransferase	Mitochondrial α-ketoglutarate dehydrogenase complex (KGDHC) can succinylate multiple mitochondrial proteins and alter their function.	[22]

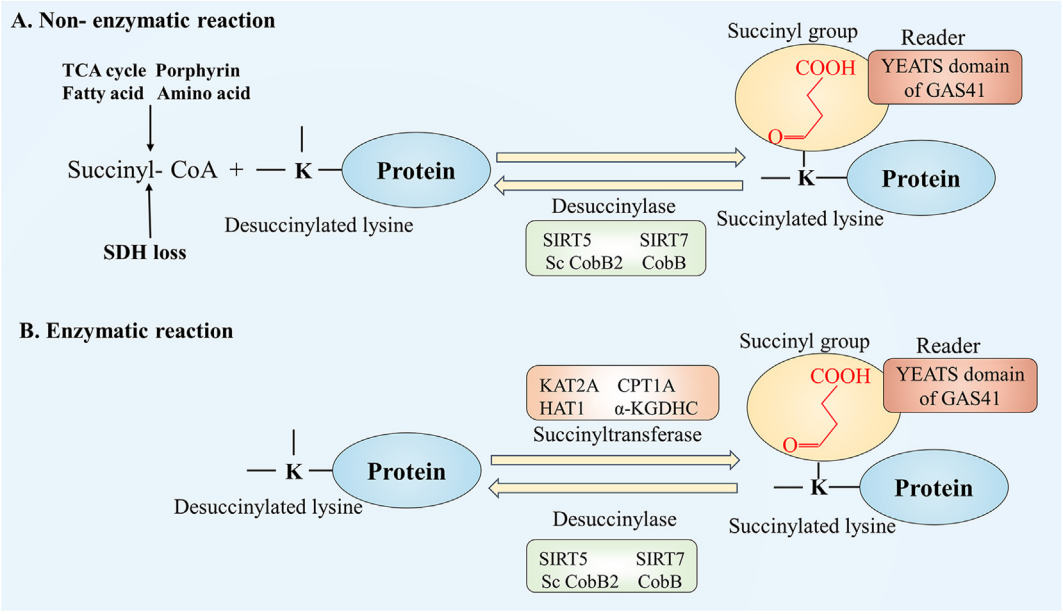

Figure 3 Enzymatic and non-enzymatic lysine succinylation. Lysine succinylation is a cellular process that a succinyl donor covalently binds succinyl groups (—CO—CH2-CH2-CO2H) to protein lysine residues through enzymatic or non-enzymatic means. **(A)** Non-enzymatic reaction: Succinyl donor succinyl-CoA participates in the succinylation modification process of the covalent binding of the lysine residues of the protein. Succinyl-CoA is mainly derived from the TCA cycle, fatty acid, amino acid, porphyrin metabolism and loss of SDH enzymes. Moreover, the dynamic balance of succinylation modification adjustment also relies on desuccinylation through desuccinylase, CobB, ScCobB2, and SIRT5/7. **(B)** Enzymatic reaction: some succinyltransferases, KAT2A, HAT1, α-KGDHC, and CPT1A also can catalyze lysine succinylation in cells. SDH, succinate dehydrogenase; TCA, tricarboxylic acid; SIRT5, sirtuin 5; SIRT7, sirtuin 7; α-KGDHC, α-ketoglutarate dehydrogenase complex; KAT2A, lysine acetyltransferase 2A; HAT1, histone acetyltransferase 1; CPT1A, carnitine palmitoyltransferase 1A; GAS41, glioma amplified sequence 41; YEATS, Yaf9, ENL, AF9, Taf14, Sas5 domain.

Enzymatic lysine succinylation

In addition to the non-enzymatic Ksuc related to succinyl-CoA and succinyl metabolites, some researchers have found and identified enzyme-catalyzed Ksuc in cells (Fig. 3).

In 2015, Gibson et al found that α-ketoglutarate dehydrogenase complex (α-KGDHC) could act as a transsuccinylase to succinylate numerous mitochondrial proteins, including the TCA cycle-related enzymes, and the related protein function was altered in an α-ketoglutarate-dependent manner. Importantly, the activity of α-KGDHC depends on the E2k subunit, which has higher activity compared with succinyl-CoA.[22] Subsequently, Wang et al reported that α-KGDHC could bind lysine acetyltransferase 2A (KAT2A), a histone succinyltransferase, and then KAT2A could succinylate histone H3 on lysine 79 (H3K79), which mainly occurs near the transcriptional start site of genes. Additionally, tyrosine 645 (Y645) in KAT2A can selectively interact with the nuclear α-KGDH complex to generate local succinyl-CoA. Mutating Y645 in KAT2A to alanine or preventing α-KGDHC from entering the nucleus can reduce the catalytic activity of KAT2A and consequently the level of H3K79 succinylation.[23] Of note, KAT2A was also found to regulate H3K79 succinylation in the promoter region of YWHAZ (encoding for 14-3-3ζ) to increase 14-3-3ζ and YWHAZ mRNA expression, thereby preventing β-catenin degradation in pancreatic ductal adenocarcinoma (PDAC) cells.[24] This suggests that protein succinylation may be involved in carcinogenesis or cancer progression.

Wang et al found that the GAS41 (glioma amplified sequence 41) YEATS (Yaf9, ENL, AF9, Taf14, Sas5) domain shows prominent binding affinity for H3K122 in a protonated histidine residue, and the coenrichment of GAS41 and H3K122suc on the p21 promoter was observed at pH 6.0—7.0. When the pH value increased to 7.4, the binding of GAS41 to H3K122suc significantly decreased and there was instead an increase in H3K122cr (croton acylation) and H3K122ac (acetylation), indicating that the GAS41 YEATS domain is a pH-dependent reader of Ksuc on histones.[25] In addition, Kurmi et al found that carnitine palmitoyltransferase 1A (CPT1A) possesses lysine succinyltransferase (LSTase) activity *in vivo* and *in vitro*. They observed that 171 lysine sites on 101 proteins (out of 550 total lysine sites on 247 proteins) were hypersuccinylated in WT-CPT1A cells. Enolase 1 was among the most prominently succinylated proteins, and the modification reduced the enolase enzymatic activity in cells. Subsequently, they found that mutating CPT1A Gly710 (G710E) selectively inactivated carnitine palmitoyltransferase (CPTase) activity but not LSTase activity, and that mutation of CPT1A G710E could decrease enolase activity and promote cell proliferation under glutamine depletion without affecting the cellular succinyl-CoA levels. These results suggest that CPT1A, as an LSTase, regulates the enzymatic activity of protein and cell

metabolism in a manner independent of its CPTase activity.[26] CPT1A has also been found to regulate the succinylation of S100A10 at lysine residue 47 (K47) in gastric cancer cells, and the K47 succinylation of S100A10 led to stabilization of the protein by inhibiting its ubiquitylation and subsequent proteasomal degradation through the proteasome.[27]

Consequently, in addition to non-enzymatic Ksuc by succinyl-CoA, Ksuc can also be accomplished via other short-chain acyl-coenzyme metabolites, resulting in changes in the cellular metabolism and cell signaling.[28] A better understanding of enzymatic Ksuc will be necessary to generate effective drugs that can modulate the Ksuc level in cells.[29]

Enzymatic desuccinylation

During the subsequent in-depth studies of the succinylation modification, several negatively regulated desuccinylases were discovered. In 2013, Colak and co-workers identified CobB as a sirtuin2-like bacterial lysine deacetylase, the first identified prokaryotic desuccinylase with deacetylation and desuccinylation activities.[30] Similarly, another sirtuin-like protein, ScCobB2, was also characterized as a specific desuccinylase in *Streptomyces coelicolor*.[31] Meanwhile, sirtuin 5 (SIRT5)[13] and sirtuin 7 (SIRT7)[14] were identified as desuccinylases in eukaryotes (Fig. 3).

A large number of studies indicate that SIRT5 acts as an NAD^+-dependent lysine deacylase, catalyzing the sequence-selective desuccinylation of various histone succinyl sites and playing an important role in diverse cellular physiological processes. For example, conserved main chain hydrogen bonds formed by the succinyl lysine (0), +1, and +3 sites are essential for the recognition of SIRT5 and succinyl peptides.[32,33] The SIRT5 gene encodes four SIRT5 protein isoforms, namely $SIRT5^{iso1-4}$, in human beings. $SIRT5^{iso1-3}$ are localized in the mitochondria, whereas $SIRT5^{iso4}$ is mainly localized in the cytoplasm. Among them, $SIRT5^{iso1}$ has higher deacylase activity than $SIRT5^{iso2-4}$.[34]

SIRT5 also plays a crucial role in cell processes such as fatty acid metabolism and amino acid metabolism.

Studies of hepatic SIRT5 overexpression in the ob/ob mice showed that these animals had the decreased succinylation and malonylation levels, enhanced fatty acid oxidation, and improved hepatic steatosis compared to healthy mice, indicating that hepatic overexpression of SIRT5 ameliorates metabolic disorders in these mice via the desuccinylation of proteins.[35] SIRT5-mediated desuccinylation can increase the activity of the enoyl-CoA hydratase α subunit (ECHA) at K351, thereby promoting fatty acid oxidation.[36] Besides, SIRT5 can also inhibit the activity of acyl-CoA oxidase 1 (ACOX1) to affect the fatty acid β-oxidation.[37] Interestingly, increased succinylation of UCP1 (uncoupling protein 1), which is regulated by SIRT5 in brown adipose tissue, significantly reduces its activity and stability, leading to defective mitophagy, impaired mitochondria respiration, and disturbed lipid metabolism.[38]

SIRT5 is also involved in glycometabolism, where it inhibits pyruvate kinase M2 (PKM2) activity by decreasing the level of succinylation at K498, thereby inhibiting pyruvate production.[39] SIRT5 can regulate the aerobic oxidation of glucose by depriving the E1α subunit of succinyl binding, thereby inhibiting pyruvate dehydrogenase complex (PDC) activity.[40] Moreover, SIRT5 was also found to desuccinylate K547 in the succinate dehydrogenase complex subunit A (SDHA), which is involved in the TCA cycle. Additionally, knockdown of SIRT5 will cause the reactivation and hypersuccinylation of SDHA.[41]

Interestingly, SIRT5 also plays crucial roles in amino acid metabolism. On the one hand, it desuccinylates serine hydroxymethyltransferase (SHMT2) at K280 and up-regulates its activity to promote serine catabolism.[42] On the other hand, SIRT5 inhibits glutamine synthesis by desuccinylating glutaminase (GLS).[43] Succinylation is the preferred SIRT5 target in the oxidative phosphorylation pathway.[35] SIRT5 acts on protein complexes on the inner mitochondrial membrane via its affinity for cardiolipin, thereby promoting the function of the respiratory chain.[44] Deficiency of SIRT5 will lead to inhibition of mitochondrial NADH oxidation and suppression of ATP synthase activity, which greatly affects ATP production in the mitochondria.[45]

In addition, SIRT5 regulates the succinylation of 3-hydroxy-3-methylglutaryl-CoA synthase 2 (HMGCS2), a rate-limiting ketogenic enzyme both *in vivo* and *in vitro*, indicating that SIRT5 is a regulator of ketogenesis through the Ksuc of HMGCS2.[13] SIRT5 can also desuccinylate and activate isocitrate dehydrogenase 2 (IDH2) and Cu/Zn superoxide dismutase (SOD1), thereby enhancing ROS reduction and the cellular antioxidant defense.[46,47]

SIRT7 has also been described as a NAD^+-dependent histone deacetylase; however, the intrinsic enzymatic activity and the cellular function of SIRT7 remain unclear. In 2016, Li et al revealed that SIRT7 is recruited to DNA double-strand breaks (DSBs) in a poly (ADP-ribose) polymerase 1 (PARP1)-dependent manner and catalyzes the desuccinylation of H3K122, thereby promoting DSB repair and chromatin condensation.[14] However, whether other substrates can be regulated by SIRT7 requires further investigation.

Lysine succinylation in eukaryotes and prokaryotes

Evidence suggests that Ksuc can dynamically regulate carbon metabolism in both human cells and bacteria,[48] and is important for other biological activities. Therefore, further understanding and exploring the functions of Ksuc is important to better understand both the normal and pathological functions of cells. A summary of the recently reported roles of Ksuc in eukaryotes and prokaryotes, is provided in Figure 4, and Tables S1 and S2.

Lysine succinylation in eukaryotes

In this section, we review the studies published about Ksuc in eukaryotes, including animals, fungi, and plants, with a particular emphasis on human cancers and some non-cancer diseases. The previous research on Ksuc in eukaryotes has mainly focused on basic studies, including identifying Ksuc sites in different eukaryotes and determining how Ksuc affects both normal cell metabolism and pathophysiological progresses.

Lysine succinylation in animals

Only six studies about Ksuc in animals have been reported, including one study each in mice,[49] zebrafish (*Danio rerio*),[50] silkworms (*Bombyx mori*),[51] *Toxoplasma gondii*,[52] Richardson's ground squirrels,[53] and *Mythimna separata*.[54] Park et al identified that 2565 Ksuc sites on 779 proteins through systematic profiling of the mouse succinylome. Strikingly, a large fraction of SIRT5 is extramitochondrial, and Ksuc is also present on nuclear and cytosolic proteins. SIRT5 suppresses cell respiration via two protein complexes, succinate dehydrogenase and the pyruvate dehydrogenase complex.[49] Similarly, 552 Ksuc sites on 164 proteins in zebrafish (*Danio rerio*) were revealed, and Ksuc plays an important role in multiple metabolic processes in these animals, including the tricarboxylic acid cycle and carbon metabolism.[50] There were 1884 Ksuc sites on 373 mitochondrial proteins identified in the silkworm (*Bombyx mori*), with these proteins being significantly enriched in the processes of mitochondrial protein synthesis and central metabolism. Importantly, several apoptosis- and detoxification-related proteins were also succinylated, indicating the important role of Ksuc in midgut metabolism and drug resistance of silkworms.[51] In addition, 425 Ksuc sites were discovered in 147 proteins in *Toxoplasma* tachyzoites, which were enriched in epigenetic gene regulation and metabolic processes.[52]

Lysine succinylation in fungi

A total of six studies have been reported revealing the potential roles of Ksuc in fungi, including *Ganoderma lucidum*,[55] *Pyricularia oryzae*,[56] *Aspergillus flavus*,[57] *Trichophyton rubrum*,[58] *Candida albicans*,[59] and *Saccharomyces cerevisiae*.[60] In total, 742 succinylated lysine sites were identified on 382 proteins in *Ganoderma lucidum*,[55] 2109 succinylated sites on 714 proteins in *Pyricularia oryzae*,[56] 985 succinylated sites on 349 proteins in *Aspergillus flavus*,[57] 569 succinylated sites on 284 proteins in *Trichophyton rubrum*,[58] and 1550 succinylated sites on 389 proteins in *Candida albicans*.[59]

Notably, Ksuc was shown to be involved in the metabolic regulation of bioactive compounds, especially pharmacologically-active compounds, in *Ganoderma lucidum*. A total of 47 enzymes related to the biosynthesis of triterpenes and polysaccharides were found to be succinylated in this fungus.[55] Succinylation may also play a vital role in the basic metabolic regulation of *Pyricularia oryzae*. Importantly, more than 40 pathogenicity-related proteins in *Pyricularia oryzae* have been identified as succinylated proteins, indicating that succinylation is involved in the pathogenicity of this organism.[56] Moreover, succinylated proteins are involved in a variety of biological processes, being especially enriched in biosynthetic processes in *Aspergillus flavus*. Ksuc of norsolorinic acid reductase NorA (AflE) can affect the sclerotia and the aflatoxin biosynthesis of *Aspergillus flavus*.[57] *Trichophyton rubrum* is one of the most common dermatophyte species. Succinylated proteins are involved in numerous cellular processes in *Trichophyton rubrum*, such as translation, epigenetic regulation, and metabolism. It was also found that 24 proteins related to *Trichophyton rubrum* pathogenicity were succinylated.[58] Similarly, *Candida albicans* is one of the most common human fungal pathogens in immunocompromised individuals. Succinylation of proteins may play an indispensable role in regulating the TCA cycle in *Candida albicans*.[59] Thus, Ksuc may represent a target for reducing the pathogenicity of fungi.

Lysine succinylation in plants

Thirteen studies have defined the roles of Ksuc in plants, including Chinese hickory,[61] rice seeds,[11,62] rice leaves,[63] paulownia,[64] strawberry stigmata,[65] *Dendrobium officinale*,[66] *Grifola frondosa*,[67] 'Anji Baicha',[68] common wheat,[69] tomato (*solanum lycopersicum*),[70] barley,[71] and paper mulberry.[72]

The Chinese hickory was identified to have 259 Ksuc sites on 202 proteins, with enrichment in the cytosolic proteins. Strikingly, seven heat shock proteins (including heat shock protein Hsp90) with 11 Ksuc sites were among these. These HSPs were up-regulated during the grafting process, indicating that succinylated HSPs may be involved in stress tolerance of the grafted Chinese hickory.[61] Similarly, 1970 Ksuc sites on 1271 proteins were identified in response to phytoplasma infection in Paulownia witches' broom (PaWB), suggesting that succinylation may be also involved in the plant response to infection.[64]

There were 854 Ksuc sites on 347 proteins identified in developing rice seeds, with most being detected on critical enzymes or proteins involved in starch biosynthetic pathways and central carbon metabolism of developing rice seed.[11] Remarkably, another study found that 133 sites on 78 proteins were commonly modified by Ksuc or acetylated peptides among the digested embryonic proteins of rice seeds 24 h after imbibition. Together, these succinylated and acetylated proteins can regulate nearly all cellular functions.[62]

Zhou et al reported that the desuccinylation of glutathione S-transferase (OsGSTU6) and catalase (CATA) changed the activities of these enzymes in rice leaves, suggesting that desuccinylation may affect the oxidative stress process.[63] In strawberry stigmata, succinylation of the assembly polypeptide 2 (AP2) and clathrin increased from 0.5 to 2 h after pollination, suggesting that succinylation is involved in vesicle trafficking and the recognition of pollen-stigma signaling substances.[65] Interestingly, fructose-bisphosphate aldolase was succinylated at K103 and K225 in *Dendrobium officinale*, indicating a potential role for Ksuc in polysaccharides synthesis.[66] Moreover, the antidiabetic activities of GFP-W isolated from *Grifola frondosa* are associated with the protein lysine acetylation (Kac), succinylation, and crotonylation levels.[67]

'Anji Baicha', an albino tea variety, was noted to have 3530 Ksuc sites on 2132 proteins involved in photosynthesis, amino acid biosynthesis, carbon fixation, and porphyrin and chlorophyll metabolism, indicating that succinylated proteins may regulate the leaf colour variability of 'Anji Baicha' during periodic albinism.[68] Furthermore, common wheat, one of the major global cereal crops, had 330 characterized Ksuc sites in 173 proteins. Succinylated proteins appear to play a crucial role in the carbon fixation and photosynthesis

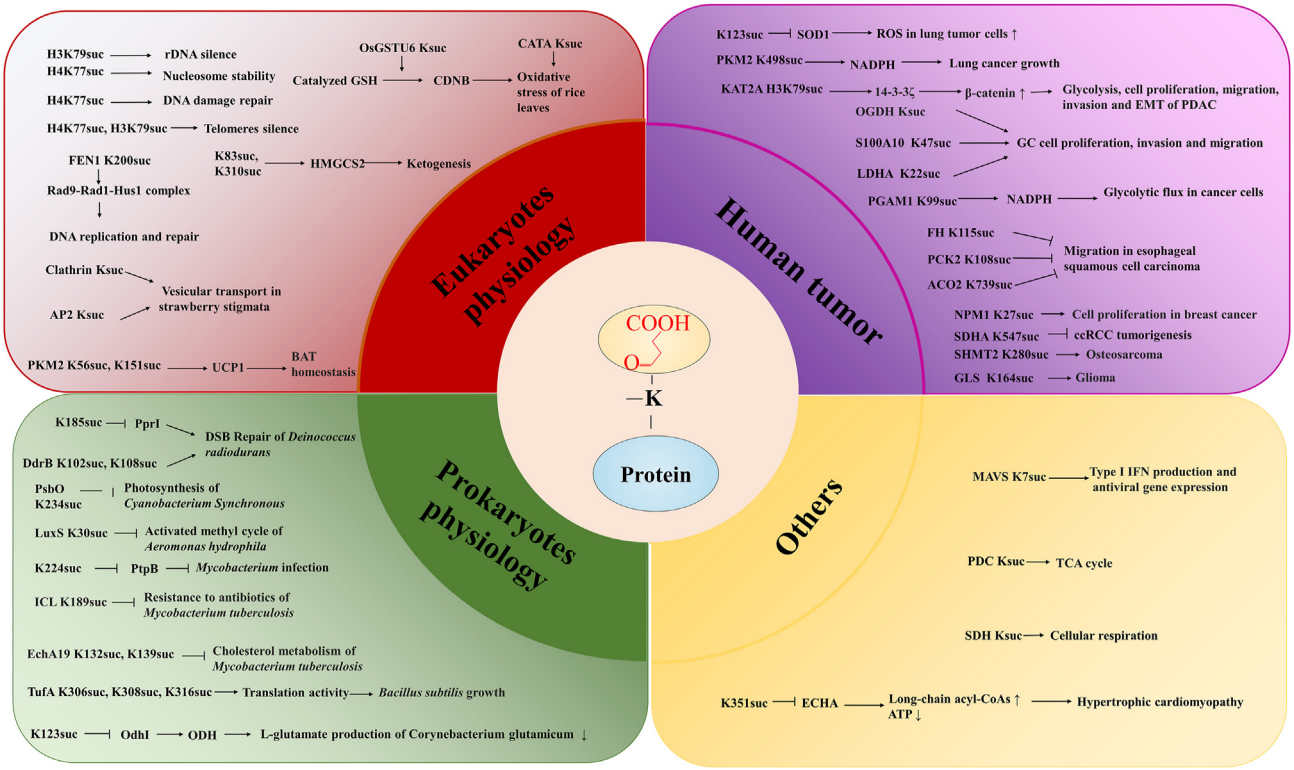

Figure 4 The physiology and pathophysiology in diverse organisms regulated by lysine succinylation. Lysine succinylation can regulate diverse cell process in normal physiology or pathophysiological process. We summarized the Ksuc regulation network in organisms in four fields: Eukaryotes physiology, prokaryotes physiology, human tumors, and others. ACO2, aconitase 2; AP2, assembly polypeptide 2; ATP, adenosine triphosphate; BAT, brown adipose tissue; CATA, catalase; ccRCC, clear cell renal cell carcinoma; CDNB, 1-chloro-2, 4-dinitrobenzene; DSB:DNA double-strand breaks; ECHA, enoyl-CoA hydratase α subunit; EMT, epithelial-to-mesenchymal transition; FH, fumarate hydratase; GC, gastric cancer; GLS, glutaminase; GSH, glutathione; HMGCS2, 3-hydroxy-3-methylglutaryl-CoA synthase 2; ICL, isocitrate lyase; IFN, interferon-gamma; KAT2A, lysine acetyltransferase 2A; LDHA, lactate dehydrogenase A; LuxS, lysines on S-ribosylhomocysteine lyase; MAVS, mitochondrial antiviral signaling protein; NADPH, nicotinamide adenine dinucleotide phosphate; NPM1, nucleophosmin; ODH, 2-oxoglutarate dehydrogenase; OGDH, 2-oxoglutarate dehydrogenase; OsGSTU6, glutathione S-transferase; PCK2, phosphoenolpyruvate carboxykinase 2; PGAM1, phosphoglycerate mutase 1; PDAC, pancreatic ductal adenocarcinoma; PDC, pyruvate dehydrogenase complex; PKM2, pyruvate kinase M2; PsbO, PSII manganese stabilized protein; ROS, reactive oxygen species; SDH, succinate dehydrogenase; SDHA, succinate dehydrogenase complex subunit A; SHMT2, serine hydroxymethyltransferase; SOD1, superoxide dismutase; TCA, tricarboxylic acid; UCP1, uncoupling protein 1.

of common wheat.[69] Similarly, 347 Ksuc sites in 202 proteins were identified in tomato, with these being mainly enriched in metabolic processes, such as chloroplast and mitochondrial metabolism, thereby affecting the metabolism and epigenetic regulation in these plants.[70]

Lysine succinylation in human cancers

Lysine succinylation has been reported to be related to various malignant tumors, including pancreatic carcinoma,[24] renal cell carcinoma,[73] gastric cancer,[27] breast cancer,[74,75] hepatocellular carcinoma (HCC),[37] esophageal squamous cell cancer,[76] colon cancer,[77] osteosarcoma,[42] bladder cancer[78] and lung cancer.[46] Notably, several succinylases and desuccinylases play crucial roles in tumor initiation and progression, such as SIRT5,[79] CPT1A,[27] histone acetyltransferase 1 (HAT1),[80] and KAT2A.[24]

SIRT5 was found to be upregulated in clear cell renal cell carcinoma (ccRCC),[41] breast cancer, colorectal cancer,[42] osteosarcoma,[42] colon cancer,[81] lung cancer[46] and downregulated in HCC.[37] SIRT5 desuccinylates the succinate dehydrogenase complex subunit A (SDHA) at K547 in ccRCC, and SIRT5 knockdown leads to hypersuccinylation and reactivation of SDHA, thereby inhibiting ccRCC cell proliferation *in vitro*.[41] Similarly, SIRT5 knockdown increases the succinylation of IDH2 and other metabolic enzymes, thereby inhibiting breast cancer tumorigenesis *in vitro* and *in vivo*.[75] Besides, SIRT5 also mediates the desuccinylation on SHMT2 at K80 to increase its activity and drive serine catabolism, thus promoting the proliferation of colorectal cancer cells and osteosarcoma cells.[42] Additionally, SIRT5 desuccinylates citrate synthase (CS) at the K393 and K395 sites and SOD1 at the K123 site, which increases their enzymatic activity, thus enhancing the growth of colon cancer cells and lung cancer cells.[46,81] Strikingly, SIRT5-mediated desuccinylation was reported to suppress acyl-CoA oxidase 1 (ACOX1) activity by inhibiting its active dimer formation *in vitro* and *in vivo*. Moreover, SIRT5

knockdown causes increased succinylation levels and ACOX1 activity, thus enhancing the oxidative DNA damage response in HCC.[37]

Carnitine palmitoyltransferase 1A (CPT1A), a lysine succinyltransferase, was overexpressed in gastric cancer.[27,82] CPT1A-mediated succinylation of S100A10 at K47 increased gastric cancer invasion and migration.[27] In addition, CPT1A also succinylates lactate dehydrogenase A (LDHA) at the K222 site, thereby reducing the binding of ubiquitinated LDHA to sequestosome 1 and inhibiting the degradation of LDHA. Increased LDHA promotes the invasion and proliferation of gastric cancer cells.[82]

It has also been reported that HAT1 expression is significantly increased in liver cancer, and HAT1 succinylates phosphoglycerate mutase 1 (PGAM1) at the K99 site.[80] This increased the enzymatic activity of PGAM1, thereby increasing the glycolytic flux in HepG2 cancer cells.[80] Similarly, KAT2A was overexpressed in pancreatic ductal adenocarcinoma (PDAC) and increased the succinylation of H3K79 in the YWHAZ promoter region encoding 14-3-3ζ, which promoted YWHAZ mRNA and 14-3-3ζ expression, thereby decreasing β-catenin degradation. Finally, high levels of 14-3-3ζ and β-catenin enhanced the glycolysis, proliferation, migration, invasion, and EMT in PDAC cells.[24] Together, these findings indicate that succinylation appears to be important for a variety of cancer types, and is involved in numerous pathways.

Lysine succinylation in non-tumor diseases

Apart from cancer, lysine succinylation is also closely related to a variety of other diseases, especially diseases related to metabolism.[49] For example, in a mouse model of non-alcoholic fatty liver disease (NAFLD), 815 succinylation sites in 407 proteins mainly located in the mitochondria and cytoplasm. This result may provide an understanding of the changes of the Ksuc levels in diverse proteins may promote the development of NAFLD.[83] Furthermore, overexpression of SIRT5 increased demalonylation and desuccinylation in the hepatic SIRT5-overexpressing ob/ob mouse model. This enhanced fatty acid oxidation and cellular glycolysis, but suppressed gluconeogenesis and hepatic steatosis through the glycolysis/gluconeogenesis pathway and the oxidative phosphorylation pathway.[35]

In Parkinson's disease, SIRT5 knockdown led to more severe nigrostriatal dopaminergic degeneration and greater sensitivity to paraquat-induced dopaminergic degeneration in mice with Parkinson's disease.[47,84] This result suggests that lysine succinylation may be a risk factor for Parkinson's disease. Interestingly, SIRT5 is up-regulated during the development of Alzheimer's disease,[84] suggesting that lysine succinylation may be a protective factor in Alzheimer's disease. Moreover, lysine crotonylation and succinylation levels are increased in the cerebral cortex of BTBR T^{+} $Itpr3^{tf}$/J (BTBR) relative to C57BL/6 J (B6) mice,[85] indicating that Ksuc may be also involved in the neuroanatomical abnormalities in BTBR mice. Xiao et al showed that 211 Ksuc sites in 170 proteins were identified in mouse brains after subarachnoid hemorrhage (SAH). Of interest, the administration of resveratrol can activate Sirt5 in SAH mice, leading to the restoration of the mitochondrial metabolism and alleviation of early brain injury.[86]

In hypertrophic cardiomyopathy, succinylated proteins mainly accumulate in the heart when SIRT5 is knocked down. Furthermore, SIRT5 knockdown inhibits ECHA (a protein involved in regulating fatty acid oxidation) activity through desuccinylation of K351, thus increasing the levels of long-chain acyl-CoAs and decreasing ATP production in the heart under fasting conditions. This suggests that SIRT5 knockdown can increase the Ksuc level and inhibit ECHA activity through the desuccinylation of Lys351, thus reducing the cardiac function, resulting in the development of the hypertrophic cardiomyopathy with aging.[36] During myocardial ischemia, SIRT5 knockdown can promote SDH activity, and aggravates cardiac ischemia-reperfusion injury,[87] suggesting that succinylation may be a risk factor for cardiac ischemia-reperfusion injury. Of note, 246 succinylation sites on 132 proteins were up-regulated and 45 succinylation sites on 117 proteins were down-regulated in the tissues of patients with atrial fibrillation (AF), indicating that succinylated proteins may affect the pathogenesis of AF in patients with heart disease.[88]

Recently, Liu et al found that 624 succinylation sites in 494 proteins were identified in mice with the gut microbiota dysbiosis that received fecal samples from patients with major depressive disorder. These proteins were mainly associated with protein translation and the synaptic vesicle cycle. Meanwhile, gut microbiota dysbiosis impacted mitochondria biological processes and the mitogen-activated protein kinase (MAPK) pathway through protein crosstalk between acetylation and succinylation.[89]

These studies indicate that lysine succinylation is involved in a wide variety of cell processes, with a specific concentration in metabolic processes and cellular physiology in eukaryotes. Further characterization of the succinylome will likely provide additional insights into the dynamic regulation of lysine succinylation, and may provide new targets for a variety of pathological processes.

Lysine succinylation in prokaryotes

Growing evidence has shown that Ksuc plays a vital role in regulating various cellular processes in both eukaryotes and prokaryotes, with a large number of studies having been performed on bacteria. The focus of most studies has been on understanding the role of Ksuc in the metabolism, drug resistance, ability to adapt to environmental changes, and pathogenicity of bacteria.

Lysine succinylation in bacteria

Studies about Ksuc have been involved in a variety of bacteria, among which those on Ksuc involvement in bacterial metabolism include *Escherichia coli* (*E. coli*),[30] *Streptomyces coelicolor* (*S. coelicolor*),[31] *Histoplasma capsulatum* (*H. capsulatum*),[90] *Corynebacterium glutamicum* (*C. glutamicum*),[91] *Vibrio parahaemolyticus* (*V. parahemolyticus*),[92] and *Mycobacterium tuberculosis* (*M. tuberculosis*).[48]

Colak and co-workers reported that lysine-succinylated proteins and succinyllysine peptides were more abundant than lysine-acetylated peptides under high-glucose conditions in *E. coli*, suggesting that glucose may have a more immediate effect on Ksuc compared with acetylation.[30]

ScCobB2-mediated lysine desuccinylation regulates the carbon metabolism and protein biosynthesis in *S. coelicolor*.[31] Our group has found that Ksuc may be associated with central metabolism in *H. capsulatum* and *M. tuberculosis*.[48,90] Similarly, acetylation and succinylation at several critical lysine residues of 2-ODHC are directly related to glutamate production in *C. glutamicum*. Importantly, Ksuc occurs more easily than acetylation when the substrates are limited.[91] Pan et al revealed that Ksuc is also involved in the protein biosynthesis and central metabolism of *V. parahaemolyticus* through succinylation and acetylation of 33 proteins in the glycolysis, gluconeogenesis, and pentose phosphate pathways, and in the TCA cycle.[92]

Recently, Ksuc was also found to be involved in drug resistance and the ability of bacteria to adapt to environmental changes. Zhou et al found that succinylated proteins can be involved in nucleic acid binding/processing processes and regulate the activities of species-specific radiation-desiccation response regulon proteins PprI and DdrB in *Deinococcus radiodurans* (*D. radiodurans*), indicating that Ksuc is involved in the extreme environmental resistance of *D. radiodurans*.[93] Additionally, our group showed that numerous metabolic enzymes and antibiotic resistance proteins, such as isocitrate dehydrogenase (IDH), KatG and KasA are succinylated in *M. tuberculosis*, suggesting that some succinylated proteins may underlie the antibiotic resistance of *M. tuberculosis*.[94] Our study also showed that Ksuc of the *M. tuberculosis* isocitrate lyase (ICL) K189 residue could tune the microbial resistance to antibiotics. Specifically, the ICL-K189E mutant strain was more sensitive to rifampicin and streptomycin compared with the wild-type strain, but this effect was not observed for isoniazid.[95] Another study on methicillin-resistant *Staphylococcus aureus* (MRSA) showed that mutations in *sucC* and *sucD*, a part of the gene cluster *mdh-sucCDAB* involved in the TCA cycle, decreased the β-lactam resistance without affecting the expression of penicillin-binding protein 2a (PBP2a). Meanwhile, increased levels of succinyl-CoA in *sucC* mutant cells significantly increased the Ksuc in the MRSA proteome.[96] This study showed that mutations in the *sucC* site increased the susceptibility of MRSA to β-lactam antibiotics, and this was accompanied by the accumulation of succinyl-CoA, which in turn affected Ksuc in the MRSA proteome.

In addition to the above findings, Ksuc has also been found to regulate the pathogenicity of bacteria such as *Porphyromonas gingivalis* (*P. gingivalis*),[97] *Pseudomonas aeruginosa* (*P. aeruginosa*),[98] and mycobacterial species.[99] Ksuc was revealed to regulate proteins related to virulence factors, including fimbriae, gingipains, PorR, and RagB in *P. gingivalis*.[97] Similarly, succinylated chitin-binding protein CbpD-PA14_53 250, elastase LasB-PA14_16 250 and transport proteins SecB-PA14_67 720, SecY-PA14_09050, and SecA-PA14_57 220 were identified to be involved in regulating the virulence of invasive proteins in *P. aeruginosa*.[98] Protein-tyrosine phosphatase B (PtpB), a vital factor involved in *Mycobacterium* infection, is negatively regulated by Ksuc and Kac at the K224 site.[99]

In addition, Ksuc was revealed to be crucial for the adaptation and fitness of *Aeromonas hydrophila* (*A. hydrophila*),[100] *P. gingivalis*,[97] and *Bacillus subtilis* (*B. subtilis*).[101] Ksuc on lysines 23 and 30 in the S-ribosylhomocysteine lyase (LuxS) regulate the abundance of AI-2 (a quorum sensing autoinducer), ultimately altering its competitiveness with another microorganism, *Vibrio alginolyticus*.[100] Additionally, 18.5% of the factors necessary for *P. gingivalis* survival *in vitro* and 12% of the fitness factors for *P. gingivalis* survival in both abscess and epithelial cell colonization environments were identified to be succinylated on various lysine residues.[97] Notably, the translation elongation factor, Tu (TufA), was found to be succinylated during vegetative growth under minimal succinate or citrate conditions in *B. subtilis*. Moreover, three succinylation mimic mutations at K306, K308, and K316 in domain-3 of TufA negatively affected *B. subtilis* growth, indicating that the succinylation level of *B. subtilis* may affect autologous growth.[101]

Lysine succinylation in other prokaryotes

Cyanobacteria are the only prokaryotes capable of oxygenation and photosynthesis, and numerous succinylation sites are found in various proteins related to photosynthesis and metabolism in these organisms.[102] The PSII manganese stabilized protein (PsbO) was discovered to be succinylated at K99 and K234 sites. Moreover, succinylation may affect photosynthesis by changing the conformation of PsbO, thus hindering the interaction between PsbO and the PSII core based on molecular dynamics simulations.[102]

Tools used to discover lysine succinylated proteins and sites

The identification of lysine succinylation sites is an important field in cellular physiology and pathology, which may provide novel information for biomedical studies and further drug development. High-throughput methods with mass spectrometry and computational tools for succinylation site prediction are now being widely used in the study of Ksuc.[103] In this section, we review these methods, including traditional experiment methods, with a focus on the computational tools being used to predict Ksuc sites.

Traditional experimental methods for identifying lysine succinylation

A proteomic tool based on sensitive immune-affinity purification and high-resolution liquid chromatography-tandem mass spectrometry (LC-MS/MS) was applied to discover novel Ksuc sites and succinylated proteins comprising the global succinylome.[61] Subsequently, high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS)[63] and Nano-LCMS/MS[11] were used to explore the succinylome. Bioinformatic analysis plays a crucial role in understanding the possible functions of succinylation. For

instance, the gene ontology (GO) annotation proteome, Kyoto encyclopedia of genes and genomes (KEGG), protein−protein interactions (PPIs) network obtained from the STRING database, and Cytoscape analysis[94] have all been used to obtain information about the possible functions of succinylation. Succinylome mapping also provides an important resource for identifying regulatory network and novel properties of succinylation.[11] These methods are making it possible to study Ksuc in an increasing number of organisms.

Computational prediction tools to predict lysine succinylation sites

It is crucial to discover succinylated proteins and their corresponding modification sites. The traditional strategies used to identify Ksuc are based on proteomic-related methods, such as LC-MS/MS.[11,61,63] These approaches are cumbersome, expensive, and time-consuming.[104] Therefore, various web tools were developed for predicting Ksuc sites (Table 2).

In 2015, Zhao et al established the first succinyl site prediction tool-SucPred, which implemented positive samples only learning (PSoL) algorithm (a special class of semi-supervised machine learning) to train the model.[105] In the same year, Xu et al used the support vector machine (SVM) with an incorporated peptide position-specific propensity into a general form of pseudo-amino acids (PseAAC) composition and developed a new prediction tool called iSuc-PseAAC.[106] Besides, They subsequently established SuccFind based on a feature selection algorithm that included an amino acid component (AAC), composition of k-spaced amino acid pairs (CKSAAP), and amino acid index (AA index).[107] Jia et al constructed two prediction tools, pSuc-Lys and iSuc-PseOpt, based on a PseAAC descriptor through a RF classifier.[108,109] The Success and SucStruct predictors were designed by López et al in 2017 and 2018, respectively. They were based on the combination of secondary structure feature (SF) *SSpre* with a bigram and decision tree (DT) algorithm. Both of them showed high sensitivity, accuracy, and a good Matthew's correlation coefficient.[110,111]

Similarly, using evolutionary features, sequence-based features, and DT classifier, Dehzangi et al designed two prediction tools, SSEvol-Suc and PSSM-Suc.[112,113] The PSuccE tool was trained to use SVM and an ensemble learning algorithm to predict succinylation sites in proteins.[114] Similarly, pSuc-PseRat and iPTM-mLys were used to predict Ksuc sites in proteins by exploiting ratios of sequence coupling and forest classifiers.[115,116] Additionally, Hasan et al developed SuccinSite, GPSuc, and SuccinSite2.0 by using amino acid patterns and properties, multi-sequence features and RF classifiers.[117−119] SuccinSite2.0 and GPSuc implement different species classifications and integrate them together.[118,119] In 2019, Huang et al developed CNN-SuccSite, enabling the search for the substrate site specificity of succinylation sites.[120] Recently, Zhu et al established Inspector using a random forest algorithm combined with feature-encoding schemes and sequence-based schemes. Inspector has high predictive mode accuracy.[121] Thapa et al developed DeepSuccinylSite, using a deep learning-based method along with embedding to identify succinylation sites through the primary structures of proteins.[122] SuccSite, which incorporates AAC and CKSAAP schemes to discover new succinylation sites[123] was developed by Kao et al. Ning et al also designed a new succinylation sites prediction tool, SSKM_Succ, by incorporating K-means clustering with a novel semi-supervised learning algorithm.[124] Subsequently, Ning and colleagues used a hybrid-learning architecture that integrated conventional machine-learning algorithms and deep-learning models into a single framework, which they named HybridSucc.[125] In 2020, Zhang et al developed an IFS-LightGBM (BO)-based prediction model, which introduced the IFS method and different feature selection approaches, and combined it with the LightGBM classifier to reduce noise and redundant information. The FS-LightGBM (BO) model performs well, with an ACC of 0.7392, MCC of 0.4771, and a F-measure of 0.7255.[126]

These methods differ in numerous aspects, including the training and test datasets, required input, preferred algorithm, whether the classifier is general or specific, and their accuracy or sensitivity. Therefore, it is necessary to understand the advantages and disadvantages of these different tools and apply the correct one(s) to generate the desired information.

The HybridSucc database currently lists 8710 identified succinylated proteins with 23 866 Ksuc sites for 13 species.[125] MDCAN-Lys[127] and LSTMCNNsucc[128] were also released for to predict succinylation sites. LSTMCNNsucc combines a convolutional neural network (CNN) and long short-term memory (LSTM) into a deep learning model for predicting succinylation sites more precisely.[128] However, it should be noted that succinylation often overlaps with other PTMs, such as acetylation, which may also be identified by like predML-Site and 'iMul-kSite'.[18,129,130] Tools that can predict the overlap with Ksuc and other PTMs, thereby revealing a complex regulatory network in cells.

From SucPred to MDCAN-Lys, there are currently 24 computational prediction tools that have been developed (Table 2). These tools provide researchers without a bioinformatics background an easy approach to discover new Ksuc sites and proteins.

Inhibitors and activators of lysine succinylation

Considering that Ksuc is involved in diverse metabolic pathways in eukaryotes and prokaryotes, finding effective inhibitors or activators of Ksuc will be crucial for pharmaceutical applications (Table 3).

Yang et al confirmed that lead (Pb) can suppress both the Kac and Ksuc processes. Further experiments showed that Pb can inhibit key enzymes in the TCA cycle, and low TCA cycle activity may reduce the acetyl coenzyme A and succinyl-CoA levels, thus inhibiting Kac and Ksuc. Importantly, the decrease of Ksuc may hinder the replacement of transition proteins during sperm elongation and the distribution of germ cells in seminiferous tubules, leading to reproductive injury.[131] Similarly, cadmium (Cd) was also found to inhibit glyceraldehyde-3-phosphate dehydrogenase (GAPDH) activity, ATP, and cAMP levels in germ cells, resulting in inhibition of Kac and Ksuc in the testis, causing

Table 2 The developed web tools or prediction model of Ksuc.

Time	Tools	Species	URL	Dataset size (Protein/succinylated)	SN(%)	SP(%)	ACC(%)	MCC	References
2015	SucPred	Generic	http://59.73.198.144:8088/SucPred/	897/2511	89.69	87.66	88.65	0.773	[105]
2015	iSuc-PseAAC	Generic	http://app.aporc.org/iSucPseAAC/	896/2521	51.07	89.42	79.94	0.431	[106]
2015	SuccFind	Generic	http://bioinfo.ncu.edu.cn/SuccFind.aspx	1044/2938	NA	NA	NA	NA	[107]
2016	iSuc-PseOpt	Generic	http://www.jcibioinfo.cn/iSuc-PseOpt	896/2521	68.80	96.48	87.44	0.7084	[108]
2016	SuccinSite	Generic	http://systbio.cau.edu.cn/SuccinSite/	2322/5004	33.93	90.67	50.22	0.251	[117]
2016	pSuc-Lys	Generic	http://www.jci-bioinfo.cn/pSuc-Lys	896/2521	26.42	82.19	42.43	0.091	[109]
2017	SucStruct	Generic	https://github.com/YosvanyLopez/	670/1782	79.46	72.86	76.08	0.5240	[110]
2017	PSSM-Suc	Generic	https://github.com/YosvanyLopez/PSSM-Suc	670/1782	81.59	82.41	81.99	0.6396	[112]
2017	pSuc-PseRat	Generic	http://ccsipb.lnu.edu.cn/pred/succ_prediction	4960/14 591	70.6	68.0	68.6	0.321	[115]
2017	SuccinSite2.0	Generic and Species-specific	https://biocomputer.bio.cuhk.edu.hk/SuccinSite2.0/	2322/5004	88.4	45.7	85.0	0.263	[118]
2018	Success	Generic	https://github.com/YosvanyLopez/Success	670/1782	85.9	80.9	83.4	0.669	[111]
2018	SSEvol-Suc	Generic	https://github.com/YosvanyLopezSSEvol-Suc	670/1782	90.9	83.7	87.5	0.75	[113]
2018	PSuccE	Generic	https://github.com/ningq669/PSuccE	2322/5009	88.6	37.5	84.5	0.204	[114]
2018	GPSuc	Generic and Species-specific	http://kurata14.bio.kyutech.ac.jp/GPSuc/	2322/5004	47.85	70.00	54.21	0.163	[119]
2019	CNN-SuccSite	Generic	http://csb.cse.yzu.edu.tw/CNN-SuccSite/	6377/18 593	84.40	86.99	86.79	0.489	[120]
2020	Inspector	Generic	NA	2322/5009	69.3	71.7	71.5	0.238	[121]
2020	DeepSuccinylSite	Generic	https://github.com/dukkakc/DeepSuccinylSite	NA	79	68.7	NA	0.48	[122]
2020	SuccSite	Generic	http://csb.cse.yzu.edu.tw/SuccSite/	1169/2509	50.43	84.32	82.93	0.18	[123]
2020	SSKM_Succ	Generic	https://github.com/yangyq505/SSKM_Succ.git	2150/4695	82.21	75.14	80.18	0.546	[124]
2020	HybridSucc	Generic and Species-specific	http://hybridsucc.biocuckoo.org/	8710/23 866	NA	NA	NA	NA	[125]
2020	IFS-LightGBM (BO)	Generic	NA	2599/5049	NA	NA	73.92	0.4771	[126]
2021	"Proposed Predictor"	Generic	NA	NA	79.8	90.2	89.10	0.629	[146]
2021	LSTMCNNsucc	Generic	http://8.129.111.5/	6377/18 593	59.16	79.57	77.89	0.2508	[128]
2021	MDCAN-Lys	Generic	NA	6377/18 593	66.81	76.75	75.97	0.2736	[127]

SN: sensitivity; SP: specificity; ACC: accuracy; MCC: matthew correlation coefficient.

Table 3 The inhibitor/activator of lysine succinylation.

Inhibitor	Model	Inhibitor/ activator	Potential target	Function	Methods	References
Rhamnolipid	*Azotobacter chroococcum*	Activator	*nifA* and *nifHDK*	Rhamnolipid enhances the nitrogen fixation activity of *Azotobacter chroococcum* by influencing lysine succinylation	4D label-free quantitative proteomic approach, and qRT-PCR	[135]
Lead (Pb)	Mouse model of Pb-induced testicular injury	Inhibitor	NA	Pb may restrain key enzymes to block the TCA cycle and that the low TCA cycle activity could reduce the contents of two important metabolites, acetyl-CoA and succinyl-CoA, to inhibit Kac and Ksuc.	Western blot, and immunofluorescence	[131]
Cadmium (Cd)	Mouse model of Cd-induced testicular injury	Inhibitor	GAPDH	Cd can restrict GAPDH activity, ATP and cAMP levels of germ cells to inhibit lysine acetylation and succinylation in the testes, inducing reproductive injuries.	Western blot, and immunofluorescence	[132]
Ethanol	Sirt3 −/− (KO) C57Bl/6J mice	Inhibitor	NA	Mitochondrial protein succinylation is reduced in ethanol -supplemented diet group.	Immunoblotting, and LC-MS/MS	[133]
Sunitinib	Renal cancer cell lines, ACHN and 786-O	Inhibitor	SIRT5	SIRT5 expression could be upregulated by sunitinib. Increased SIRT5 enhances the expression of isocitrate dehydrogenase 2 and protein stability by desuccinylation at K413.	Western blott, protein stability assay, and qRT-PCR	[134]
Sodium dichloroacetate	Human colon cancer cell line HCT116	NA	NA	179 succinylation sites on 108 proteins were up-regulated and 114 succinylation sites on 71 proteins were down-regulated in human colon cancer HCT116 cells when treated with sodium dichloroacetate.	LC-MS/MS	[77]
AUY922	Human bladder cancer cell line, 5637 (HTB-9)	NA	NA	In bladder cancer cells treated with HSP90 inhibitors	HPLC-MS/MS and Western blot	[78]

Table 3 (*continued*)

Inhibitor	Model	Inhibitor/ activator	Potential target	Function	Methods	References
				AUY922 and ganetespib, 34 histone PTM sites were identified, including H4K20suc and H3K122suc.		
Ganetespib	Human bladder cancer cell line, 5637 (HTB-9)	NA	NA	In bladder cancer cells treated with HSP90 inhibitors AUY922 and ganetespib, 34 histone PTM sites were identified, including H4K20suc and H3K122suc.	HPLC-MS/MS and Western blot	[78]
DK1-04e	Breast cancer mouse model	Inhibitor	SIRT5	DK1-04e and DK1-04am, with stronger cytotoxicity, increased succinylation more than JH-I5-2am and JH-I5-2e. And the effects of SIRT5 prodrug inhibitors on cancer cells are dependent on SIRT5 inhibition.	Nano LC-MS/MS, and immunoblotting	[75]
DK1-04am	Breast cancer mouse model	Inhibitor	SIRT5	DK1-04e and DK1-04am, with stronger cytotoxicity, increased succinylation more than JH-I5-2am and JH-I5-2e. And the effects of SIRT5 prodrug inhibitors on cancer cells are dependent on SIRT5 inhibition.	Nano LC-MS/MS, and immunoblotting	[75]

reproductive damage.[132] In addition, the Ksuc of mitochondrial proteins was decreased in mice fed with ethanol, indicating that Ksuc may be affected by ethanol.[133] It is possible that other toxic substances may exert their effects at least partly via inhibition of Ksuc.

Importantly, Abril et al revealed that SIRT5 knockdown in breast cancer cells increased the succinylation of IDH2 and some other metabolic enzymes, leading to high oxidative stress and impairing the transformation of the cells. They subsequently developed a small molecule SIRT5 inhibitor, which inhibited the transformation of breast cancer cells and obviously suppressed breast cancer growth *in vivo* in xenotransplant mouse models. This appeared to occur through inhibition of SIRT5 expression and increased Ksuc levels in both the cytoplasm and mitochondria of breast cancer cells.[75] Additionally, another group found that SIRT5 expression could be upregulated by sunitinib. Increased SIRT5 enhances the expression of IDH2 and protein stability due to a lack of succinylation at K413, which may partially contribute to sunitinib resistance in renal cell carcinoma cells.[134]

As of the writing of this review, the study of Ksuc activator is scarce. Only a study by Li et alshowed that there were 1376 Ksuc sites on 645 proteins in *Azotobacter chroococcum* (*A. chroococcum*) cells when treated with rhamnolipid (RL) for 22 h, compared with 1372 Ksuc sites on 639 proteins when treated with RL for 0 h, indicated RL may enhance the Ksuc level in *A. chroococcum*.[135]

Furthermore, 179 succinylation sites on 108 proteins were up-regulated and 114 succinylation sites on 71

proteins were down-regulated in human colon cancer HCT116 cells when they were treated with sodium dichloroacetate, which indicates that the anti-cancer effect of sodium dichloroacetate may be closely related to lysine succinylation, but with alterations in the status of various proteins rather than global upregulation.[77] Moreover, in bladder cancer cells treated with HSP90 inhibitors AUY922 and ganetespib, 34 histone PTM sites were identified as being altered by the treatment, including $H4K20_{suc}$ and $H3K122_{suc}$, suggesting that HSP90 inhibitors may exert anti-tumor effects through lysine succinylation.[78] Thus, various treatments may impact Ksuc, but further work is needed to identify specific activators.

Conclusions

In 2011, Ksuc was first identified as a previously unreported and evolutionarily conserved PTM.[12] It has since been discovered that Ksuc is involved in regulating diverse cellular functions ranging from cell metabolism[136] to tumorigenesis.[27] High-resolution LC-MS/MS made it possible to identify the whole succinylome in cells.[11] The subsequent emergence of Ksuc prediction tools has facilitated the discovery and annotation of new Ksuc sites.[105]

A single protein may have multiple PTMs simultaneously.[137] Therefore, the crosstalk between Ksuc and other PTMs, such as Kac, in regulating protein function should be emphasized. Moreover, human diseases often have a multi-factor phenotype that cannot be easily explained by a single PTM. It is currently unknown whether the crosstalk among different PTMs can affect diseases progression and which factors determine which PTM is dominant in certain cell processes. It is likely that various PTMs may interact with each other to more dynamically regulate cellular functions.

However, the current knowledge of succinylation is still limited. For instance, there are only a few succinyltransferases and desuccinylacylases of Ksuc that have been discovered so far, and the identification and study of the full complement of succinyltransferases and desuccinylacylases is crucial to reveal their functions. Moreover, despite the increasing evidence that succinylation functions in regulating diverse functions during both disease progression and normal physiological function through histones, it remains unclear how Ksuc affects these processes via non-histone proteins. Furthermore, although a few endogenous and exogenous inhibitors or potential activators of succinylation have been described, additional research will be crucial to provide a theoretical basis for the future development of relevant small molecular drugs to treat diseases arising due to dysfunctional Ksuc.

In the future, the discovery of more succinylation sites and proteins that undergo Ksuc will be made through proteomics studies and/or using computational tools. Efforts should be devoted to understanding how human physiological processes and pathological processes are mechanistically regulated by Ksuc. Once the basic information is understood, the research results can be translated into the clinic for a variety of applications.

Author contributions

LX and JX conceived the study protocol. GZ, JZ, JG, XL, DL, SW and LX participated in the literature search and the data collection. GZ, JZ, XL, DL, SW, JG and LX analyzed the data. GZ, JG and LX drafted the manuscript. GZ, JG, JX and LX revised the manuscript. All authors read and approved the final manuscript. GZ, JZ and LX contributed equally to this study.

Conflict of interests

Authors declare no conflict of interests.

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Appendix A. Supplementary data

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