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Review Article

Role of succinylation modification in thyroid cancer and breast cancer

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Abstract: The incidence of thyroid cancer and breast cancer is increasing year by year, and the specific pathogenesis is unclear. Posttranslational modifications constitute an important regulatory mechanism that affects the function of almost all proteins, are essential for a diverse and well-functioning proteome and can integrate metabolism with physiological and pathological processes. In recent years, posttranslational modifications, which mainly include metabolic enzyme-mediated protein posttranslational modifications, such as methylation, phosphorylation, acetylation and succinylation, have become a research hotspot. Among these modifications, lysine succinylation is a newly discovered broad-spectrum, dynamic, non-enzymatic protein post-translational modification, and it plays an important regulatory role in a variety of tumors. Studies have shown that succinylation can affect the synthesis of thyroid hormones, and the regulation of this post-translational modification can inhibit the apoptosis and migration of thyroid cancer cell lines, and promote breast cancer cell proliferation, DNA damage repair and autophagy-related regulation. However, the specific regulatory mechanism of succinylation in thyroid cancer and breast cancer is currently unclear. Therefore, this article mainly reviews the research progress of succinylation modification in thyroid cancer and breast cancer. It is expected to provide new directions and targets for the prevention and treatment of thyroid cancer and breast cancer.

Keywords: Thyroid cancer, breast cancer, posttranslational modification, succinylation, succinyl coenzyme

Introduction

Thyroid cancer (TC) and breast cancer (BC) are the two most common malignant tumors in women, and their incidence is increasing each year [1-4]. Studies conducted over the past few years have found that patients with a previous incidence of BC or TC exhibit a significantly higher overall risk of TC or BC from second primary tumor, respectively [5-8], which indicates a two-way and potential causality relationship between BC and TC. In addition, the development of these two tumor types has common etiological characteristics [9], such as hormonal factors (both TC and BC are regulated by the hypothalamic-pituitary axis), genetic factors (e.g., mutations in the tumor suppressor gene PTEN cause Cowden syndrome (CS), which is associated with a high incidence of BC and TC) and environmental and treatment-related factors [10]. Moreover, most types of TC have a good prognosis, but some types of TC are resistant to surgery and radioactive iodine therapy (RAI) [11, 12] and have a poor prognosis [13]. For example, poorly differentiated thyroid carcinoma (PDTC) shows poorly differentiated and higher metastatic tendency and the patient prognosis is poor [14, 15]. This therapy resistance makes the treatment of TC difficult. Similarly, BC is not only the malignant tumor with the highest incidence in women in the world, but also the main cause of female cancer deaths [16]. Its recurrence and metastasis rates are high, and the prognosis for metastatic BC is poor [16-19]. Therefore, it is necessary to further explore new diagnosis and therapy targets for TC and BC.

Protein modification refers to chemical modification after protein biosynthesis, also known as posttranslational modification (PTM). Studies have shown that PTMs play important regulatory roles in biochemical reactions and that the modification of proteins in different states leads to different functions [20, 21]. Hundreds of PTMs have been identified, and these include methylation, acetylation, phosphorylation, ubiquitination, glycosylation, and succinylation, etc. [22-24]. Among these PTMs, DNA methylation represents a basic epigenetic modification that can regulate chromatin structure and gene transcription [25]. Many diseases, including cancer, display abnormal methylation patterns [26]. DNA methylation inhibitors have been used to block methylation-dependent gene silencing for the treatment of hematopoietic tumors and restoration of the expression of silenced developmental genes [27]. Similarly, histone acetylation modifications (HAMs) affect cell function and are mainly mediated by histone acetyltransferase (HAT) and histone deacetylase (HDAC), and the HAMs are essential for the development and prognosis of BC and TC [28, 29]. In addition, phosphorylation is also one of the most widely studied PTMs, and is associated with colorectal cancer (CRC) [30], glioblastoma (GBM) [31], lung cancer [32, 33], BC [34, 35]. Therefore, PTMs are essential for the regulation of biological protein functions and cancer progression.

Succinylation is a newly discovered protein PTM that is widely present in cells and can participate in a variety of life activities by regulating protease activity and gene expression [36, 37]. Studies have shown that succinylation can change the processes of enzymes and metabolic pathways (particularly mitochondrial metabolic pathways) [38-40] and thus plays a variety of important roles in cell metabolism [41, 42], such as in tricarboxylic acid circulation, the electron transport chain, glycolysis, ketone body formation, fatty acid oxidation, and the urea cycle [43, 44]. Succinylation is also related to many diseases, such as diseases of the liver, heart, lung and other organs [21]. In addition, increasing evidence shows that succinylation modulators can promote or inhibit a variety of cancers by regulating the succinylation level of substrate targets [45-47]. For example, carnitine palmitoyl transferase 1A (CPT1A) promotes BC cell proliferation through succinylation enolase 1 [48], and promotes gastric cancer (GC) cell metastasis through the succinylation of S100A10 [46, 49]. In addition, lysine acetyltransferase 2A (KAT2A) can upregulate 14-3-3ζ through its succinyltransferase activity to promote the proliferation, migration and invasion of human pancreatic ductal adenocarcinoma (PDAC) cells [50]. The complex of KAT2A and α-ketoglutarate dehydrogenase (α-KGDH) acts as a histone H3 succinyltransferase and promotes the proliferation and development of glioma cells [51]. In addition, succinylation regulators may promote the malignant progression of renal clear cell carcinoma (ccRCC) by regulating the infiltration of immune cells and the methylation of RNA N6-methyladenosine (m6A) [52]. In contrast, the downregulation of SIRT5 (a desuccinylase) is related to the succinylation of ACOX1, an increase in its activity and the oxidative DNA damage response in hepatocellular carcinoma (HCC) [45]. And in lung cancer recombinant human superoxide dismutase-1 (SOD1) inhibits the growth of lung tumor cells through a novel posttranslational regulation of succinylation and SIRT5-dependent desuccinylation [53]. In addition, in osteosarcoma cells, the inactivation of SIRT5 can cause serine hydroxymethyltransferase (SHMT2) to enzymatically downregulate and eliminate cell growth under metabolic stress [54].

In short, succinylation can promote the occurrence of BC, gastric cancer, renal clear cell carcinoma, glioma and other tumors, and has a tumor suppressor effect on liver cancer, lung cancer and osteosarcoma cells (see **Table 1** for details). However, relatively few studies have investigated the role of succinylation in the thyroid and breast, but the available evidence shows that the regulation of succinylation can inhibit the apoptosis of TC cells and promote their migration. The PTM also has important functions in BC cells, such as promoting proliferation and mediating DNA damage repair. Therefore, studying the role of succinylation modification in the occurrence and development of TC and BC has also become a new focus of cancer research, but there are few reviews in this field. Therefore, we summarized the regulatory role of succinylation modification in these two tumors, in order to determine new targets for the treatment of TC and BC patients.

Table 1. Succinylation expression in various tumors, gene symbol, impact, K_{succ} sites, regulatory factors of succinylation and inhibitor

Tumor	Gene Symbol	Impact in Tumors	K_{succ} Sites	Regulatory Factors of Succinylation	Inhibitor	References
Gastric Cancer	S100A10	Promote cancer cell invasion and metastasis	K47	CPT1A/SIRT5	CHX	[46]
Lung Cancer	SOD1	Inhibits cancer cell growth	K123	Mutation/SIRT5	Hygromycin	[53]
Clear cell renal cell Carcinoma	AGER/IL20RB/SAA1	Immune cell infiltration and m^6A methylation	HNRNP (A2B1/C/G), LRPPRC/EIF3B	CPT1A/SIRT5/ SIRT7/KAT2A	PD-1	[52]
Glioma	KAT2A (Tyr645Ala)	Tumour cell proliferation and development	H3K79	Succinyl-CoA/α-KGDH	IPTG	[51]
Hepatocellular Carcinoma	ACOX1	Inhibit the progression of liver cancer	K89R/K437R/K488R/ K500R/K537R/K637R	SIRT5	Streptomyces hygroscopious	[45]
Breast Cancer	GLS	Breast cell proliferation and tumor formation	K164	SIRT5	Leupeptin	[48]
Osteosarcoma	SHMT2	Inhibits the proliferation of cancer cells	K280	SIRT5	Nicotinamide	[54]
Thyroid Cancer	SDHx/PTEN	Inhibit thyroid cancer cell apoptosis and promote migration	SDHD-G12S/SDHD-H50R	SDH/Succinic acid	FAD/NAD	[100]

Basic characteristics and function of succinylation

The succinylation of proteins involves succinyl group donors, such as succinyl-CoA [37, 55, 56], and refers to the process of the covalently bonding of a succinyl group to a lysine residue of a protein through enzymatic or nonenzymatic methods [57-59]. In 2004, succinylation was first discovered in *Escherichia coli*, followed by eukaryotes [37, 58, 60]. To date, approximately 20,000 succinylated gut microbial peptides have been identified in the human gut flora [61]. Succinylation is also a common PTM in prokaryotes and eukaryotes, and this process mainly occurs in the cytoplasm and nucleus [57, 62].

In the cytoplasm, succinylation mainly occurs in mitochondria, and the mitochondrial enzyme a-ketoglutarate dehydrogenase complex can control succinylation. Succinylation, which is also the key mitochondrial process of energy production [63], directly couples the cyclic metabolism of tricarboxylic acid (TCA) with changes in the charge, structure and activity of a protein through succinyl-CoA to participate in different cellular processes and can link metabolism with protein function to regulate the body's metabolic activities [38]. In addition, G protein-coupled receptors (GPCRs) also play an important role in the production of mitochondrial adenosine triphosphate (ATP) [64]. GPRG1 in the GPCR family is also known as succinate receptor 1 (SUCNR1), is extensively involved in human cell metabolism [65] and activates intracellular calcium mobilization and dendritic cytokine-dependent proinflammatory cytokines [66], and thereby activates immune cells and enhances inflammation [67].

In contrast, in the nucleus, succinylation can occur in more than one-third of nucleosomes and in both histone and nonhistone lysine residues [68]. Lysine succinylation (Kcuss) provides the lysine group with two negative charges and thereby causes changes in protein properties. A succinyl group has a larger spatial structure than an acetyl group. Therefore, succinylation has a greater impact on the structure and function of a protein [37]; for example, the α-ketoglutarate dehydrogenase (α-KGDH) complex is located in the nucleus of human cell lines, interacts with lysine acetyltransferase 2A (KAT2A, also known as GCN5) by binding to the promoter region of the gene [51], and participates in gene transcription regulation [68]. KAT2A also acts as a succinyltransferase and succinylates histone H3 on lysine 79, with a maximum frequency around the transcription start sites of genes. Preventing the α-KGDH complex from entering the nucleus, or expression of KAT2A (Tyr645Ala), reduces gene expression and inhibits tumour cell proliferation and tumour growth [51].

Regulatory factors of succinylation

Succinylation is regulated by both nonenzymatic and enzymatic factors. It was initially thought that succinylation is a PTM of proteins controlled by nonenzymatic factors [69, 70]. Similar to acetyl-CoA, succinyl-CoA is an inherently reactive short-chain coenzyme A thioester that can maintain the stability of the internal environment in the mitochondrial matrix over a low concentration range (0.1-0.6 mM). The mixing of succinyl-CoA with albumin or isocitrate dehydrogenase (ICDH) can increase succinylation in a PH and dose-dependent manner [39, 57, 58]. Protein acylation in mitochondria may be an alkaline chemical event promoted by high concentrations of active acyl-CoA in the mitochondrial matrix [39, 69]. In summary, the nonenzymatic process of succinylation is mainly controlled by the concentration of succinyl-CoA in mitochondria, pH, and protein parameters [39, 71, 72].

In-depth research on succinylation has revealed that the level of succinylation is mainly regulated by succinyl donors, succinyltransferases and desuccinylases. The succinyl donor succinyl-CoA is mainly derived from mitochondria [58] and is produced by amino acid metabolism or the TCA cycle [73]. Succinyl-CoA is mainly produced by regulation of the TCA cycle polyprotein complex α-ketoglutarate dehydrogenase complex (KGDHC), which consists of three components, E1k [α-ketoglutarate dehydrogenase (KGDH) (EC 1.2.4.2)], E2k [dihydrolipoyl succinyltransferase (EC 2.3.1.61)] and E3 [dihydrolipoyl dehydrogenase (EC 1.8.1.4)] [73]. KGDHC can indirectly change the level of succinylation (such as regulating the level of succinyl-CoA) or directly participate in the succinylation process. For example, in yeast, the mutation and loss or activation of the E1k gene can change the level of succinylation [58]. Moreover, KGDHC can mediate succinylation under

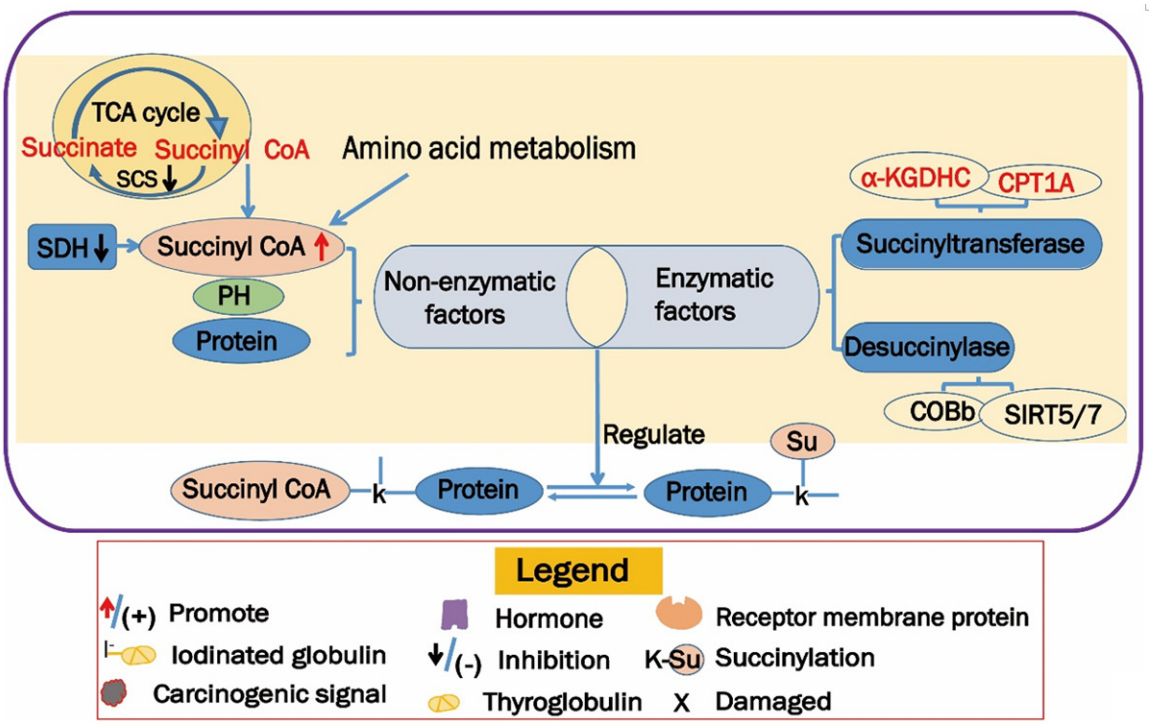

Figure 1. The process of succinylation and its simple regulatory factors. This figure mainly shows that the succinyl donor succinyl-CoA in the protein participates in the succinylation modification process of the covalent binding of the lysine residues of the protein through enzymatic or non-enzymatic regulatory factors. Among them, succinyl-CoA is mainly derived from the TCA cycle, amino acid metabolism and down-regulation of SDH enzymes. The dynamic balance of succinylation modification adjustment mainly relies on enzyme control factors (succinylase α-KGDHC, CPT1A and desuccinylase COBb, SIRT5/7) and non-enzymatic control factors (succinyl-CoA, pH value and protein Parameters, etc.) regulation.

a variety of conditions. For example, the compound I inhibitor rotenone can inhibit respiration by inhibiting aconitase and reduce the ketoglutarate in KGDHC to reduce the concentration of succinyl-CoA in mitochondria [74]. Etelvino, et al., indicated that the amino acids are used for the synthesis of heme, an important source of succinyl-CoA, and this process is also mediated by KGDHC [75]. If the TCA cycle cannot provide sufficient succinyl-CoA, glutamine is deaminated to α-ketoglutarate and is converted by α-ketoglutarate diacid dehydrogenase (KDH) into succinyl-CoA for the synthesis of heme [76]. In addition, the succinic acid is a key regulator of hypoxia [77], which leads to tumor formation and congenital inflammatory diseases. Another transferase with lysine succinyltransferase activity in mammalian cells is carnitine palmitoyltransferase 1A (CPT1A) [48], as has been confirmed in gastric cancer (GC) [46]. CPT1A is a mitochondrial enzyme that uses succinyl-CoA as a substrate without changing the succinyl-CoA levels to increase lysine succinylation in cells [48]. In summary, the above-mentioned succinyl donors and succinyltransferases, including the Ros-α-ketoglutarate dehydrogenase complex (ROS-KGDHC) [57, 58] and carnitine palmitoyl glyceride 1A (CPT1A) [48], exert positive regulatory effects on succinylation [58].

In addition, desuccinylases, such as COBb (the first succinylase found in prokaryotes) [37] as well as SIRT5 [72] and SIRT7 [78] in the sirtuin family, exert inhibitory effects on succinylation modification. Previous studies have shown that the consumption of SIRT5 can impair the function of complex II (SDH) and fatty acid β-oxidation, which indicates that succinylation inhibits mitochondrial enzymes [40, 79] and thereby affects mitochondrial metabolism. In summary, the replacement of factors during succinylation can help us understand the transformational processes of succinylation, which will benefit the diagnosis and treatment of diseases (**Figure 1**).

Role of succinylation in thyroid physiology and thyroid cancer

Regulatory effect of succinylation on thyroid physiology

The main functions of the thyroid gland, which is the body's largest endocrine organ, are to synthesize thyroid hormone, maintain the body's metabolism and function, and promote the growth and development of infants and young children [80-82]. The protein precursor of thyroid hormone is thyroglobulin (TG) [83, 84]. Thyroid hormone is synthesized through the coupling of iodine and tyrosine and is completed by TG proteolysis [85]. Iodinated TG, which is a macromolecule, is stored in the follicular cavity of the thyroid, and this process is accompanied by the activation and release of thyroid-stimulating hormone (TSH) [86].

Tarutani O and Dunn JT et al. [87, 88] showed that sodium dodecyl sulfate (SDS) treatment can decompose the iodine protein obtained by the human body into three subunits (S-19, S-27 and S-12). A large amount of 19S, 27S or 12S thyroglobulin is closely related to the ability of tumors to accumulate radioactive iodine; therefore, TG is used as a potential biomarker for the diagnosis of TC [89]. In addition, some scholars have studied the dissociation effect of SDS and succinic anhydride on thyroid 27S iodide, and the results have shown that the succinyl dissociation mode of 27S iodide is basically the same as that of SDS treatment [87], and the succinylation can affect the dissociation of iodoprotein subunits [90, 91]. Tanini A et al. [90] showed that after extensive succinylation, the 26000-Da peptide isolated from the 19S protein in the human thyroid gland can unfold and disrupt noncovalent bond interactions and thereby affects the synthesis of thyroxine [90]. In addition, previous studies have found that extensive succinylation can induce the cleavage of 12S thyroglobulin, increase the affinity of thyroglobulin for membrane receptors [92], and thereby affect the interaction between hormones on the thyroid membrane and their specific receptors [93]. In summary, the effects of the chemical modification of succinyl on the thyroid mainly include regulating the synthesis of thyroid hormones and regulating the affinity of thyroglobulin for membrane receptors, but the specific mechanisms of action remain unclear and need to be further explored (**Figure 2**).

Roles of succinylation in thyroid cancer

Studies have shown that TC may be caused by abnormal TSH secretion caused by TG mutation. Moreover, the TG levels after thyroidectomy are significantly related to the prognosis of papillary thyroid carcinoma and follicular carcinoma and can predict tumor recurrence and metastasis [86]. Therefore, the factors that affect TG changes (such as succinylation) deserve our attention.

Previous studies have shown that SDH, also known as mitochondrial complex II (the SDH enzyme, also known as mitochondrial complex II), is a heterotetrameric protein composed of four subunits, namely, SDHA, SDHB, SDHC and SDHD, and participates in the electron transport chain and the tricarboxylic acid cycle [94, 95]. In the TCA cycle, the biallelic loss of genes encoding the subunits of the SDH complex can lead to succinate and succinyl-CoA accumulation [96]. Therefore, SDH participates in the regulation process of succinylation. Succinic acid is a metabolite of the TCA cycle, and its accumulation is downregulated due to the presence of SDH and transmits "carcinogenic" signals from the mitochondria to the cytoplasm. Once in the cytoplasm, succinic acid inhibits HIF-α prolyl hydroxylase (PHD) and thereby stabilizes HIF-1α under normoxic conditions [97]. Thus, succinate can increase the expression of genes that facilitate angiogenesis, metastasis, and glycolysis, which ultimately leads to tumor progression.

CS is a Madelina autosomal dominant genetic disease, and patients with CS are prone to BC, TC and other cancers [98]. TC is one of the main components of CS, which is usually related to a germline mutation of PTEN. Germline variants of different subunit genes encoding SDH (SDHBB-D) are found in 10% of PTEN mutation-negative CS/CS-like (CSL) individuals [99, 100]. Notably, in 2012, some scholars noted that SDHB-D mutations coexist with germline PTEN mutations, including PTEN (mut+) patients or PTEN and (mut+)/SDH (var+) carriers, in approximately 6-8% of CS/CSL individuals. In addition, individuals with SDH (var+) alone exhibit a higher prevalence of TC [99]. It has been established that germline SDHD vari-

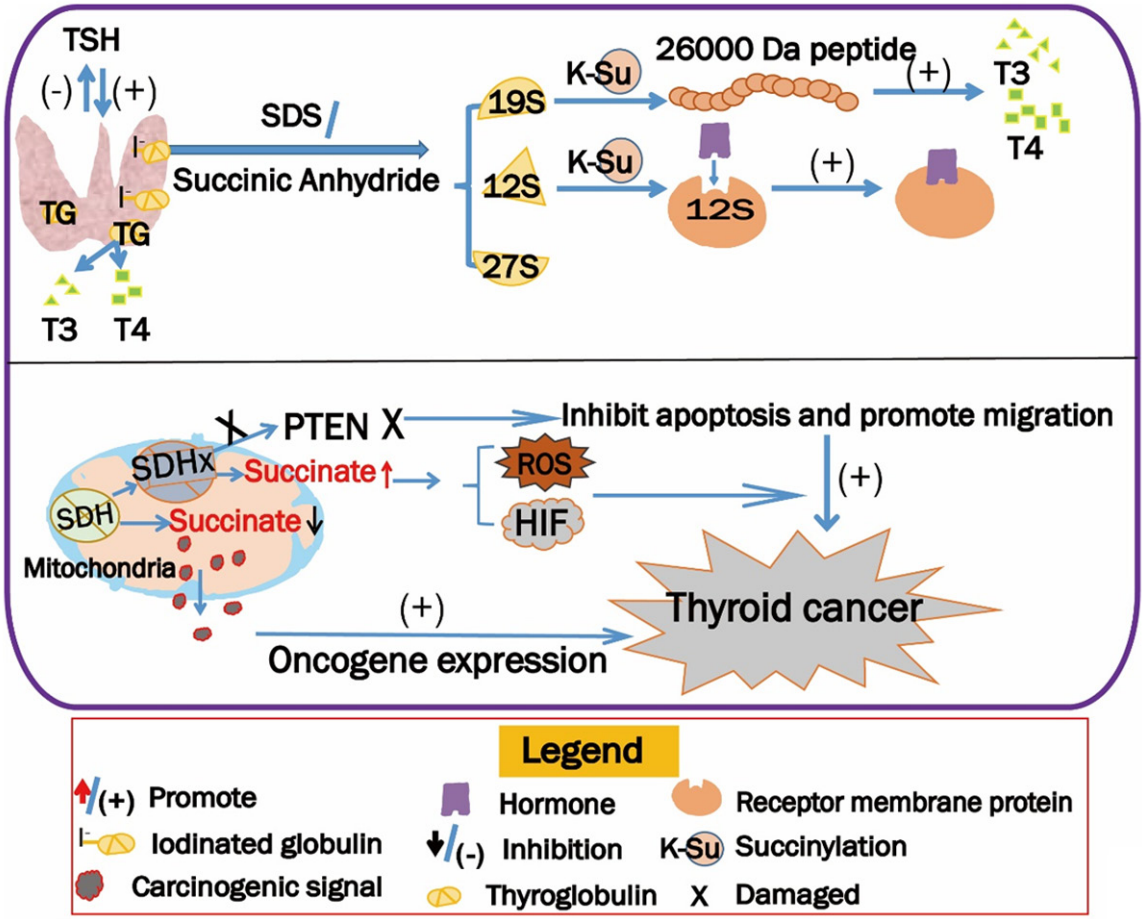

Figure 2. The pathological and physiological effects of succinylation on thyroid. This figure shows that under physiological conditions, the protein precursor thyroglobulin (TG) of thyroid hormone is mainly regulated by TSH. Iodinated TG can be decomposed into three subunits, 19S, 12S and 27S. After succinylation, it can promote the synthesis of thyroxine and affect the interaction of hormones and their specific receptors on the thyroid cell membrane. Under pathological conditions, the presence of SDH enzyme will down-regulate the accumulation of succinic acid in mitochondria, which can transmit "carcinogenic" signals from mitochondria to the cytoplasm and promote the occurrence of thyroid cancer.

ants and somatic SDHD/B changes are associated with hereditary and sporadic TC, respectively [101-103].

The variants G12S and H50R are both located in the signal peptide of the SDHD preprotein. Previous studies have found that these two variants (G12S and H50R) may cause mislocalization of the SDH complex in the mitochondrial membrane or change the cleavage site of the signal peptide [104]. In papillary thyroid carcinoma and follicular carcinoma cell lines, SDHD-G12S and SDHD-H50R change the subcellular localization of PTEN and impair the function of PTEN, which results in inhibition of the apoptosis of TC cells and promotion of the migration of TC cells [105]. In addition, during the process of mitochondrial metabolism, the succinic acid that accumulates from the dysfunctional SDH complex can not only act as a second messenger to activate HIF signals but also drive the generation of intracellular ROS. The additional ROS stress accompanied by SDHx mutations can inhibit the apoptosis of TC cells and promote tumorigenesis [97, 105, 106], and the HIF signaling pathway can also be regulated by the AKT and mTOR signaling pathways downstream of PTEN to promote tumorigenesis [107]. In addition, NQO1 is a protein containing FAD, and the presence of excessive FAD can lead to activation of the AKT and MAPK signal transduction pathways [108]. In SDHx mutant cells, the combination of NQO1 and p53 leads to loss of

NQO1 function, which leads to a reduction of p53 protein [99], and this reduction affects the occurrence of tumors. In summary, in CS/CSL tumorigenesis, the occurrence of TC may occur through the regulation of HIF1α, p53 and PTEN signal transduction by mitochondrial metabolism. The interaction between SDH and PTEN can lead to the activation of a variety of tumor-mediated signaling pathways (such as AKT, mTOR, and MAPK) and thereby promotes the occurrence of TC.

In contrast, in 2016, ED Accordi et al. conducted a study of a large Brazilian family and found that our common papillary thyroid carcinoma (PTC) is not associated with SDHx mutations [109], but because the author only studied one family, we believe that the insufficient sample size may have affected the final result. In 2017, further research conducted by W. Yu et al. revealed that SDHD-G12S and SDHD-H50R mutations can also increase the nuclear phosphorylation of AKT, which in turn affects the phosphorylation of the subsequent transcription factor FOXO3a (the main regulator of autophagy). This phosphorylation leads to the downregulation of autophagy [110], which suggests that SDHD plays a role in the pathogenesis of differentiated TC related to autophagy. The latest research has shown that missense mutations in the SDH complex are also considered the cause of differentiated TC. In addition, Compared with the more common multifocal paraganglioma found in carriers of SDHD mutation (PGL-1), carriers of SDHB mutation (PGL-4) are more likely to develop malignant diseases and extrahepatic tumors, including renal cell carcinoma and TC [111, 112]. Therefore, the correlation between various types of thyroid and SDHx mutations remains controversial and needs to be further explored by researchers.

In addition, in 2017, Lai X [113] et al. found that succinyl-CoA ligase [GDP-forming] subunit beta is a protein biomarker that can be used for the diagnosis of thyroid follicular carcinoma and for distinguishing follicular carcinoma from follicular adenoma. Among the intermediate metabolic enzymes, isocitrate dehydrogenase (IDH1/2) mutations have been found in a variety of cancers, including TC [114], and these newly discovered carcinogenic metabolic lesions may be new anticancer treatments of selective targets. For example, in mice, by downregulating the mitogen-activated protein kinase (AMPK) pathway and inhibiting epithelial-mesenchymal transition, the SRC inhibitor SKI-606 (bosutinib) can significantly prevent the dedifferentiation, vascular invasion and lung metastasis of TC cells [115]. Therefore, oral SKI-606 can be considered for the clinical treatment of patients with refractory TC.

In summary, in TC, succinyl-CoA ligase [GDP-forming] subunit β can be used as a biodiagnostic marker for follicular TC, whereas SDH subunit mutations are related to various types of TC. Therefore, we speculate that each mutant protein subunit of SDH may be a biomarker for distinguishing different types of TC. However, relatively few studies have investigated the succinylation of goiter, and further research is needed (**Figure 2**).

Role of succinylation in the physiology of the mammary gland and breast cancer

Regulatory effect of succinylation on mammary gland physiology

The mammary gland is a symbolic organ of mammals, and its development mainly occurs after birth and consists of three consecutive stages: puberty, pregnancy and lactation. The main function of this gland is the production of milk [116, 117]. The macronutrients and biologically active ingredients found in milk play an important role in the nutrition of breastfed infants and dairy products. These ingredients include some physiologically related compounds, such as vitamins, peptides, neuroactive compounds and hormones [118]. Among the components, succinyl-CoA and acetyl-CoA, among others, will increase linearly with increases in the pantothenic acid (RPP) supplement dose [119].

As early as 1958, researchers found that the activities of succinyl oxidase and cytochrome oxidase are increased in the mammary gland tissue of lactating rats, and both activities exhibited similar activity patterns: the activities gradually increase during pregnancy and childbirth and enter the stable phase during lactation, and this phase is followed by a significant increase in the activity of succinyl oxidase [120, 121]. Based on these findings, succinylation may have a certain regulatory effect during pregnancy, childbirth and lactation in mam-

mals. In addition, the proteins in cow's milk (particularly casein) are widely regarded as a good carrier for the delivery of various biologically active compounds (including minerals). Succinylation is one of the most common chemical modification techniques for enhancing the mineral binding capacity of casein. The addition of minerals to a succinylated protein may change the physical, chemical and biochemical properties of the protein [122]. PH-adjusted succinylation can improve the storage properties of emulsions by increasing the surface charge density and amphiphilic balance. Therefore, succinylation has great potential to adjust the relationship between the structure and properties of protein-based products [123]. In addition, in milk, the prepared succinylated milk proteins can be used for preparation of the milk protein-vitamin A (Vit A) complex, and consumption of this type of milk can prevent the occurrence of Vit A deficiency [124]. In summary, the succinylation may play an important regulatory role in milk secretion and mammary physiology (**Figure 3**).

Pathological functions of succinylation in breast cancer

At present, with the rapid development of proteomics technology, BC proteomics and PTM research, including phosphorylation, acetylation, methylation, and succinylation, have gradually become a research hotspot. Liu C et al. studied the effect of lysine succinylation on BC for the first time. These researchers found multiple succinylation sites in BC and discovered a new mechanism, namely, the pentose phosphate pathway (PPP) and the endoplasmic reticulum protein processing pathway can be regulated by lysine succinylation modification of its core enzyme [125]. Previous studies have shown that lysine succinylation is involved in energy metabolism, and the PPP is the main pathway of glucose catabolism. It is known that the PPP is significantly regulated in cancer cells, and it has been determined that changes in the succinylation levels in this pathway follow a specific pattern. The inhibition of aerobic respiration enhances the glycolytic pathway. To meet the rapid growth of cancer cells, the PPP is activated, and the nicotinamide adenine dinucleotide (NADPH) content is increased [126]. Most of the related reactions in this process are reversible, which indicates that the PPP balance can be controlled by succinylation. In advanced cancers, the PPP proteins show a significant increase in succinylation, which indicates that the activation of lysine succinylation may be the main cause of changes in protein expression. In addition, the TCA cycle produces and consumes succinic acid (the intermediate substrate for succinylation of lysine), whereas the PPP uses the intermediate component of glycolysis to produce NADPH (a reducing substrate), which is essential for oxidative stress [125-127]. Therefore, it has been speculated that during the regulation of glucose metabolism activated by breast tumors, the balance between the production and consumption of succinic acid can lead to the complex regulation of succinylation and protein expression, which in turn affects the occurrence and development of BC.

Gao X et al. [128] systematically studied the succinylation and acetylation modification of all proteins in invasive ductal carcinoma using proteomics technology and found that the modification level of most proteins in BC tissue was significantly higher than that in normal paracancerous tissues. In addition, a bioinformatics analysis revealed that highly succinylated proteins are significantly enriched in histone H2A.X complex, and nucleophospholipid 1 (NPM1) may be a key determinant [128]. Yu, et al., showed that the H2A.X complex can form dynamic lesions at DNA break sites under different DNA damage conditions [129]. Damage to DNA activates H2A.X, and activated H2A.X recruits many other proteins to form protein foci for mediating DNA damage repair [130]. This process can be regulated by protein modification [131-133]. Therefore, the hyperacetylation and succinylation of proteins in the H2A.X complex may affect DNA damage repair by regulating the formation of protein foci in BC [128].

In addition, L. Polletta et al. found that the immunoprecipitation of SIRT5 (lysine desuccinase) and glutaminase and the inhibition of SIRT5 can lead to an increase in the succinylation of glutaminase and promote BC [134, 135]. Glutamine, which is the most abundant nonessential amino acid, can be used as a stored form of glutamate and ammonia and is important for the tricarboxylic acid cycle [136]. Therefore, in tumor cells that use glucose mainly through glycolysis, the metabolism of gluta-

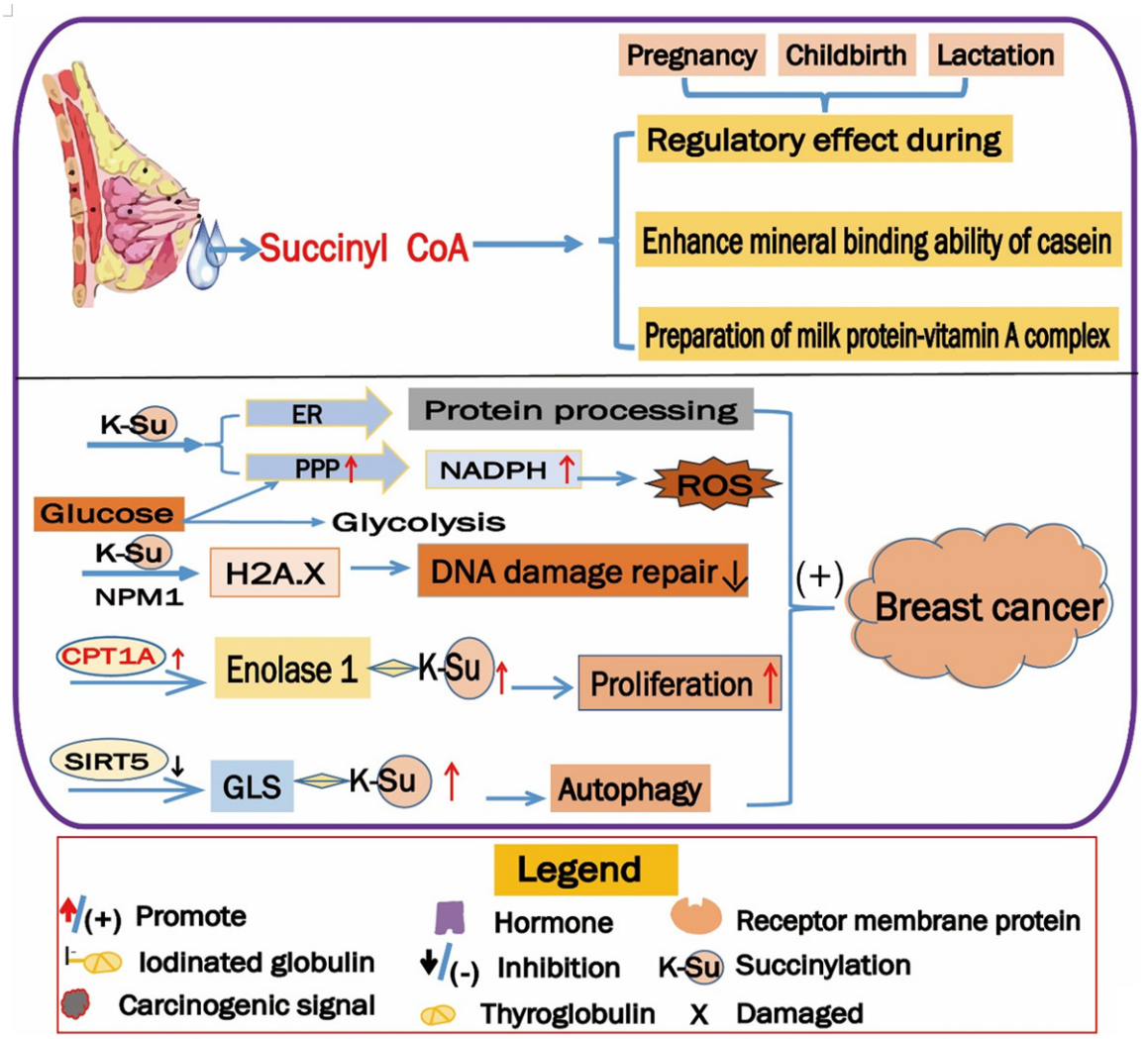

Figure 3. The pathological and physiological effects of succinylation on breast. The figure shows that under physiological conditions, the mammary, as an organ for secretion of milk, is regulated by succinylation during pregnancy, childbirth and lactation. Moreover, succinyl-CoA in milk can be modified by succinylation to enhance the binding capacity of casein minerals. Under pathological conditions, succinylation promotes the occurrence of breast cancer by stimulating oxidative stress, inhibiting DNA damage repair, and promoting the proliferation and autophagy of breast cancer cells.

mine is accelerated, and glutamine represents an important source of energy [137-139]. According to previous studies, SIRT5 can play a cancer-promoting effect in BC through the desuccinylation substrate glutaminase (GLS) [135], and SIRT5 can regulate ammonia by regulating the metabolism of glutamine in BC cells. The production and ammonia-induced autophagy [134] indicate that the metabolism of ammonia in BC is related to the regulation of succinylation. In addition, the latest study found that SIRT5 is highly expressed in human BC and is associated with a poor prognosis [140, 141]. It is worth noting that SIRT5 can be also used as a biomarker for the response of triple-negative BC to anthracycline taxane neoadjuvant chemotherapy [141]. In addition, Fu XL et al. identified the expression level of succinylated proteins. The succinic acid in the endoplasmic reticulum (ER) pathway was significantly increased, which indicated that succinylation may occur in ER proteins, and the abnormal expression of ER-related proteins may be destroyed in BC tissues [125, 142]. Other studies include that CPT1A can promote the proliferation of BC cells by the succinylation of enolase 1 [48].

In summary, there are relatively few studies on succinylation in BC. However, current studies

reveal that succinylation can be a cancer-promoting factor in BC: succinylation not only promotes BC cell proliferation but also affects the repair of DNA damage and its energy metabolism process through its regulators. Therefore, we can reasonably assume that the specific regulatory role of succinylation in BC will become a new research direction in targeted therapy for BC (**Figure 3**).

Summary and prospects

Succinylation participates in a variety of physiological and pathological processes, as well as involved in the occurrence and development of a variety of tumors. Despite extensive work has been performed to investigate the expression pattern, functional diversity, regulatory mechanism and pathophysiology of succinylation in the breast and thyroid organs, the systematic review is rare in this field. Therefore, this review focuses on basic characteristics and function of succinylation, regulatory factors of succinylation, and pathological role of succinylation in thyroid and breast cancer (**Figures 1**-**3**). We hope that we can provide a basic, systemic and summarized knowledge to this field, advocating researchers play more attention on the pathophysiological role of succinylation in the development and progression of thyroid and breast cancer, which may provide novel targets for the clinical diagnosis and treatment of these diseases.

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Disclosure of conflict of interest

None.

Abbreviations

ACOX1, acyl-CoA oxidase 1; α-KGDH, α-ketoglutarate dehydrogenase; ATP, Adenosine triphosphate; BC, Breast cancer; BRCA, breast cancer; CRC, Colorectal cancer; CPT1A, Carnitine palmitoyltransferase 1A; CS, Cowden syndrome; ccRCC, clear cell renal cell carcinoma; CHX, cycloheximide; ER, Endoplasmic reticulum; FAD, flavin adenine dinucleotide; GBM, Glioblastoma; GPCRs, G protein-coupled receptors; GC, Gastric cancer; GLS, glutaminase; HAMs, Histone acetylation modifications; HAT, Histone acetyltransferase; HDAC, Histone deacetylase; HCC, hepatocellular carcinoma; ICDH, Isocitrate dehydrogenase; KGDHC, Ketoglutarate dehydrogenase complex; Ksucc, Lysine succinylation; KAT2A/GCN5, Lysine acetyltransferase 2A; KGDHC, α-ketoglutarate dehydrogenase complex; KGDH, α-ketoglutarate dehydrogenase; LC, lung cancer; KDH, α-ketoglutarate diacid dehydrogenase; NADPH, Nicotinamide adenine dinucleotide; NPM1, Nucleophospholipid 1; NAD, Nicotinamide adenine dinucleotide; PDTC, Poorly differentiated thyroid carcinoma; PTM, Posttranslational modification; PGL-1, SDHD mutation carriers; PGL-4, SDHB mutation carriers; PPP, Pentose phosphate pathway; PTEN, phosphatase and tensin homolog; TC, Thyroid cancer; RAI, Radioactive iodine therapy; ROS-KGDHC, Ros-α-ketoglutarate dehydrogenase complex; RPP, Pantothenic acid; SUCNR1, Succinate receptor 1; SCS, Succinyl-CoA synthetase; SHMT2, Serine hydroxymethyltransferase; SDH, Succinate dehydrogenase; SIRT5, Silent information regulator 5; SIRT7, Silent information regulator 7; SDS, Sodium dodecyl sulfate; SDHx, Succinate dehydrogenase genes; SDHA, Succinate dehydrogenase A; SDHB, Succinate dehydrogenase B; SDHC, Succinate dehydrogenase C; SDHD, Succinate dehydrogenase D; TCA, Tricarboxylic acid cycle; TG, Thyroglobulin; TSH, Thyroid stimulating hormone; Vit A, Vitamin A.

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