The glycogen cargo receptor STBD1: from biology to disease targeted therapies

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Abstract

Glycophagy is a novel selective autophagy characterized by glycogen degradation via the lysosomal enzyme acid α-glucosidase (GAA). Starch-binding domain-containing protein 1 (STBD1) is a glycogen cargo receptor that mediates glycophagy through binding glycogen transport into lysosomes. STBD1-dependent glycophagy has garnered considerable interest in the pathology community, because its dysregulation causes multiple diseases, with cancer being the most serious. Notably, deletions and/or mutations in STBD1 promote tumorigenesis. In this review, we first summarized the current understanding of STBD1, including its structure, subcellular localization, tissue distribution, and biological functions. Next, we examined the roles and molecular mechanisms of STBD1-dependent glycophagy in various diseases (e.g., Pompe disease, Parkinson's disease, cardiac diseases, and cancer). Based on available research, we discussed the promising function and future of STBD1, including its potential application as a therapeutic target in glycogen-related diseases. We recommend using real-time diagnostic tools to observe the progression of STBD1-mediated glycophagy and gain further insight on the mechanism of action. Such details are needed to investigate new avenues for glycogen-related disease therapy.

The glycogen cargo receptor STBD1: from biology to disease targeted therapies

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Abstract

Glycophagy is a novel selective autophagy characterized by glycogen degradation via the lysosomal enzyme acid α-glucosidase (GAA). Starch-binding domain-containing protein 1 (STBD1) is a glycogen cargo receptor that mediates glycophagy through binding glycogen transport into lysosomes. STBD1-dependent glycophagy has garnered considerable interest in the pathology community, because its dysregulation causes multiple diseases, with cancer being the most serious. Notably, deletions and/or mutations in STBD1 promote

tumorigenesis. In this review, we first summarized the current understanding of STBD1, including its structure, subcellular localization, tissue distribution, and biological functions. Next, we examined the roles and molecular mechanisms of STBD1-dependent glycophagy in various diseases (e.g., Pompe disease, Parkinson's disease, cardiac diseases, and cancer). Based on available research, we discussed the promising function and future of STBD1, including its potential application as a therapeutic target in glycogen-related diseases. We recommend using real-time diagnostic tools to observe the progression of STBD1-mediated glycophagy and gain further insight on the mechanism of action. Such details are needed to investigate new avenues for glycogen-related disease therapy.

Keywords: STBD1, Glycophagy**,** Pompe disease, Cardiovascular disease, Cancer

Abbreviations:

STBD1: Starch-binding domain-containing protein 1; GABARAPL1: γ-aminobutyric acid receptor-associated protein-like 1; LIR: LC3 interacting region; ER: Endoplasmic reticulum; GS: glycogen synthase; GDE: glycogen debranching enzyme; NMT: N-myristoyltransferase; OSER: organized smooth ER; MAM: mitochondria-associated membranes; GAA: acid α-glucosidase; cAMP:cyclic adenosine monophosphate; PKA: protein kinase A; G6PC: glucose-6-phosphatase-α· SIRT1: silencing information regulatory factor 2 related enzymes 1; FoxO: forkhead box-containing protein O; PI3K: phosphatidylinositol 3-kinase; PP2A: protein phosphatase 2A; TSC 2: tuberous sclerosis complex 2; PPARα: proliferator-activated receptor α· G6P: glucose-6-phosphate; G6PC: glucose-6-phosphatase; AMPK: AMP-activated protein kinase; Akt: protein kinase B; mTOR: Mammalian target of rapamycin; BAT: Brown adipose tissue; WAT: white adipose tissue; LD: lipid droplets; GN: glycogenin; GYS1: Glycogen synthase 1 UPR: the unfolded protein response; BafA1: bafilomycin A1; ERT: enzyme replacement therapy; DM: Diabetes mellitus; DCM: diabetic cardiomyopathy; NRVMs: Neonatal rat ventricular myocytes; AA: Asiatic acid; PD: Parkinson's disease; ATGs: autophagy-associated genes; BRCA: breast invasive carcinoma; COAD: colon adenocarcinoma; HNSC: head and neck squamous cell carcinoma; LUSC: lung squamous cell carcinoma; BLCA: bladder urothelial carcinoma; STAD: stomach adenocarcinoma; LIHC: liver hepatocellular carcinoma; CTRP: Therapeutics Response Portal

Introduction

Starch-binding domain-containing protein 1 (STBD1, also known as Genethonin-1 [1]) was originally discovered in a large-scale differential expression screening for genes specific to skeletal muscles [2]. Encoded by a novel gene termed GENX-3414 [3], STBD1 is a receptor for glycogen-selective autophagy and regulates cellular glucose homeostasis [4].The protein contains an N-terminal hydrophobic region and a C-terminal carbohydrate-binding module (CBM20), with are two specific motifs (AIM and leucine-zipper). Intriguingly, STBD1 interacts with γ-aminobutyric acid receptor-associated protein-like 1 (GABARAPL1) through the AIM motif [5]. Studying this interaction led to the conclusion that STBD1 as a receptor is involved in glycophagy and that STBD1-mediated glycophagy accelerates lipid droplet formation in brown fat [6,7].

STBD1-mediated glycophagy is associated with various diseases, including Pompe disease, Parkinson's disease, diabetic cardiomyopathy, ischemic myocardial injury, and cancer [8-12]. However, the exact role of STBD1 in cancer is unclear. Recent studies have found that inhibiting STBD1-mediated glycophagy facilitates tumor growth via activating glycolysis, the TCA cycle, and the pentose phosphate pathways [12]. Hence, targeted glycolysis inhibition has potential as a personalized therapeutic approach for cancer patients with low STBD1 expression or STBD1 motif mutations.

In this review, we first introduced the structure, subcellular localizations, and tissue distribution of STBD1 and specifically described its participation in the glycophagy. Next, we described STBD1-related biological functions. We also summarized diseases associated with STBD1-mediated glycophagy and reviewed research on their mechanisms. Finally, we examined the possible clinical roles of STBD1 and future research directions.

2. Overview of STBD1

2.1 Protein structure

STBD1 is a 39 kDa protein encoded by the *GENX-3414* located at the 4q24±q25 on the chromosome. Highly conserved in all organisms [5,13]. STBD1 is involved in glycogen metabolism and cellular transport [14]. Its 358 amino acids include a conserved N-terminal hydrophobic sequence and a C-terminal carbohydrate-binding module that binds glycogen. Notably, STBD1 is characterized by two specific motifs: the Atg8 interacting motif (AIM, also known as the LC3 interacting region [LIR)] and a leucine-zipper motif (**Figure 1A**). The topology of STBD1 from PROTTER (http://wlab.ethz.ch/protter/start/) equally illustrates its protein structure (**Figure 1B**).

The AIM motif (residues 200-206) is a common structural domain of the selective autophagy receptor, that binds to the LC3/GABARAP family of autophagy-modifying proteins [5,15]. GABARAPL1 combines with the AIM motif in STBD1 to promote glycophagy spontaneously. Deletion or mutation of AIM abolishes the intrinsic function of STBD1 [16,17]. Regrettably, the function of the leucine zipper motif (residues 69–90) is currently unknown and a subject for future research (**Figure 1A, B**).

Both the conserved N-terminal structure and CBM20 are indispensable to STBD1 functions. The hydrophobic N-terminal structure (first 24 amino acids) allows STBD1 to target the endoplasmic reticulum (ER). Partial deletion of this N-terminal structure causes STBD1 to distribute diffusely throughout the cytoplasm, thus disabling its regulation of glycogen metabolism [14]. Moreover, STBD1 undergoes ubiquitination, a process that requires the N-terminus [5,18]. CBM20 is crucial for STBD1 stability and its interaction with glycogen-related proteins. The CBM20 domain binds glycogen-related carbohydrates (e.g., amylose, amylopectin, and polyglucosans) and glycogen-related proteins, such as glycogen synthase (GS), glycogen debranching enzyme (GDE), and Laforin [18]. Mutation of a conserved tryptophan residue (W293) in CBM20 eliminated the ability of STBD1 to bind the carbohydrate amylose and caused the protein to degrade rapidly, pointing to the domain's importance in maintaining stability [18]. Clarifying the functions of N-terminal structure and CBM20 provided novel insight into how STBD1 binding partners are regulated (**Figure 1A, B**).

A study using ProfileScan to determine STBD1 post-translational modifications and protein structure sites found multiple potential phosphorylation sites, including four protein kinase C sites and 12 casein kinase II sites [19]. In addition, one potential N-linked glycosylation site and five potential myristoylation sites in the predicted amino acid sequence [3,20]. As expected, the N-terminal hydrophobic segment of STBD1 is the main region involved in myristoylation [21], where N-myristoyltransferase (NMT) adds myristate (a saturated 14-carbon fatty acid) to the exposed N-terminal glycine residue (position 2, Gly2) [22]. N-myristoylated STBD1 affects ER-mitochondrial binding and mitochondrial morphology [21]. STBD1 myristoylation is critical to its localization, facilitating ER retention of STBD1 and leading to the formation of an organized smooth ER (OSER). However, the absence of myristate promotes protein targeting of mitochondria-associated membranes (MAM). Non-myristoylated STBD1 alters mitochondrial network morphology and ER-mitochondria contacts, inducing prominent mitochondrial fragmentation and clustering [21]. Collectively, these findings on STBD1 structure improves our understanding of the protein's biological function and pathogenic mechanisms.

As already mentioned, STBD1 orthologs are highly conserved across organisms. (**Figure 1C**). The homologous genes for human STBD1 (hSTBD1; 358 amino acid residues) and mouse Stbd1 (mStbd1; 338 amino acid residues) showed approximately 60% identity. Rat Stbd1 is 88% and 63% identical to the mouse and human homologs respectively[5]. However, protein sequence analysis revealed that CBM20 does not appear to exist in other vertebrates (such as fish and birds), although the domain is ancient and present in bacteria and archaea [13].

2.2 Subcellular localizations and tissue distributions

Subcellular STBD1 was found in the ER, plasma membrane, cytosol, lysosomes, extracellular space, Golgi apparatus, endosomes, nuclei, peroxisomes, and mitochondria, according to date from the COMPARTMENTS (*https://compartments.jensenlab.org/Search)* (**Figure 2A**). STBD1 mRNA is mainly distributed in the skeletal muscle, tongue, liver, adipose tissue, and heart muscle based on correlational data from the Human

Protein Atlas (*http://www.proteinatlas.org/)*(**Figure 2B**). Multiple studies using techniques such as confocal microscopy and immunodetection have verified STBD1 subcellular localization in the transverse tubule, sarcoplasmic reticulum, ER, MAM, and perinuclear regions [3,14,21].

Notably, STBD1 also function as an ER-resident protein. The N-terminal hydrophobic region anchors the protein to the ER and deleting this region causes STBD1 to distribute throughout the cytoplasm. N-myristoylation, a common post-translational modification of the N-terminus, mediates STBD1 subcellular localization in the ER [21]. Recent studies have revealed that the addition of myristate facilitates protein retention in the ER and depletion of myristate prone proteins targeting the MAM. STBD1 knockout results in distortion in the integrity of hepatic MAM, associating with insulin resistance [23].

Intriguingly, immunofluorescence detected increased endogenous STBD1 expression in FL83B mouse liver cells and Rat1 fibroblasts. STBD1 is mainly concentrated in perinuclear structures [24,25], which are co-localized to varying degrees with the lysosomes, ER, and Golgi apparatus. A plausible explanation of this co-localization is that STBD1 overexpression affects the trafficking of subcellular organelles, promoting the aggregation of organelles into expanded perinuclear structures containing STBD1. However, STBD1 function in the perinuclear region are remains unclear and requires further investigation.

3. STBD1 functions as a receptor in glycophagy

Macroautophagy/autophagy is an evolutionarily conserved degradation pathway that maintains cellular homeostasis. As we are known that autophagy begins with the formation of a bilayer membrane called a phagophore. The phagophore subsequently engulfs cytoplasmic materials and closes to form an autophagosome. The autophagosome then rapidly transports its cargo to autolysosomes for degradation [26,27]. Cargo broken down by autolysosomes include damaged proteins, dysfunctional organelles, and pathogens [28]. Currently, we know of three major autophagy types: macroautophagy, microautophagy, and chaperone-mediated autophagy [29]. Autophagy dysfunction has been implicated in disease pathology progression [30].

Initially considered a non-selective bulky degradation process induced by hypoxia, energy or amino acid deprivation, radiation, and drugs, autophagy is now understood to display highly selective properties [30]. Autophagy receptors initiate selective autophagy and link cellular material to the autophagy compartment through simultaneous interactions with cargo and Atg8-or LC3/GABARAP-like proteins on autophagosomes [31,32], thus eliciting specific autophagosome formation [33]. The vast majority of selective autophagy has been identified based on the types of targeted intracellular materials. These include mitochondria (mitophagy), protein aggregates (aggrephagy), pathogens (xenophagy), ribosomes (ribophagy), ER (ER-phagy or reticulophagy), nuclear envelope (nucleophagy), liposomes (lipophagy), ferritin (ferritinophagy), lysosomes (lysophagy), and glycogen (glycophagy) [27,31,33-41].

Glycophagy is a novel selective autophagy pathway to be identified. After encapsulation by lysosomes, glycogen is degraded by lysosomal acid α-glucosidase, resulting in the release of free α-glucose [42]. Glycogen-rich lysosomes were first discovered in the 1970s and called "glycogen lysosomes" [43]. Subsequently, lysosomal GAA degradation of glycogen was termed glycogen autophagy [44], and discovered to play a critical role in the early stage of human birth. When the hepatic glycogen levels decreased sharply, glycogen autophagy is activated, causing glycogen lysosomes to increase in number, total volume, and activity. The process produces non-phosphorylated glucose, which acts with glucose 6-phosphate (G6P) to counteract postnatal hypoglycemia. In addition, the two forms of glucose participate in other metabolic pathways, ultimately achieving glucose homeostasis in the neonatal period [45].

The process of glycogen transport to the lysosome has not been extensively explored, but research isolating STBD1 from human skeletal muscle found that the protein transports glycogen to lysosomes and thus is a novel receptor mediating glycophagy. The interaction between STBD1 and Atg8 family member GABARAPL1 is indispensable in regulating STBD1-dependent glycophagy [5,18]. Profiling of the STBD1 AIM motif interacting with GABARAPL1 showed that AIM ($_{200}$HEEWEMV$_{206}$) is mainly located in a predicted disordered region of STBD1 and fits the consensus AIM sequences of other cargo-specific proteins, such as p62 and Nix (mediating aggrephagy and mitophagy) [46,47]. In addition, STBD1 can co-localize with

GABARAPL1, but not with macroautophagy marker LC3, implying that glycogen autophagy is different from macroautophagy [5]. The identification of STBD1 as the cargo-binding protein that delivers glycogen to lysosomes in an autophagic pathway led to naming the process "glycophagy".

Glycophagy is mainly activated through the cyclic adenosine monophosphate (cAMP)/ protein kinase A (PKA), glucose-6-phosphatase (G6PC) / silencing information regulatory factor 2 related enzymes 1 (SIRT1) / forkhead box-containing protein O (FoxO) and Ca^{2+} signaling pathways. Its primary inhibitory pathways are phosphatidylinositol 3-kinase (PI3K)/ mammalian target of rapamycin (mTOR) and protein kinase B (Akt)/FoxO signaling [41,45,48-50]. Positive regulation of the cAMP/PKA signaling pathway and negative regulation of the PI3K/mTOR axis, converge at the common target (e.g., protein phosphatase 2A, PP2A). This convergence regulates GAA formation and affects glycophagy [45]. Increased cAMP, (especially cAMP/ATP) promotes glycophagy through activating AMP-activated protein kinase (AMPK) and inhibiting mTOR. When AMPK is downregulated, mTOR is activated to impede glycophagy [51]. Knocking out tuberous sclerosis complex 2 (TSC 2) reactivated mTOR in muscles of Pompe disease mice and inhibited glycophagy [49]. In another independent pathway, G6PC deletion suppresses peroxisome proliferator-activated receptor α (PPARα), downregulates SIRT1 and increases ATG acetylation, reduces ATG12-ATG5 binding, and downregulates FoxO signaling, impairing glycophagy [50]. Conversely, elevated G6PC expression normalizes glycophagy via restoring SIRT1 and FoxO3 signaling [50]. Actually, insulin activates Akt to inhibit FoxO1 and FoxO3 binding to the GABARAPL1 promoter, blocking GABARAPL1 transcription and glycogen entry into lysosomes [52]. Of note, calcium levels influence the activation of glycogen-hydrolyzing acid glucosidase and acid mannose 6-phosphatase, facilitating non-phosphorylated glucose transport from the lysosome to the cytoplasm and speeding glycophagy progression [45] (**Figure 3**). In addition, some drugs affect glycophagy by regulating lysosomal GAA activity. Adrenaline (a β-adrenergic agonist) and rapamycin (a mTOR inhibitor) upregulate cardiac and hepatic GAA activity in vivo [48], and propranolol (a β-adrenergic receptor antagonist) decreases hepatic GAA activity in neonatal rats [53]. Ciclopirox olamine can upregulate STBD1 activity and promote glycophagy, inhibiting cervical cancer cell proliferation [54]. In summary, glycophagy is catalyzed in a controlled manner by a diverse array of signaling pathways and drugs.

4. Biological functions

4.1 STBD1 facilitates the formation of lipid droplets in brown adipose tissue

Brown adipose tissue (BAT) is a primary source of thermogenesis and maintaines body temperature through non-shivering heat generation [55,56]. Glycogen particles were observed in BAT using transmission electron microscopy in the mid-twentieth century [57,58]. Experiments on adult rats and rabbits showed that BAT has higher glycogen content and lower fat content than white adipose tissue (WAT) [59]. Glycogen particles have also been observed in human BAT [60].However, glycogen formation, activation, and function in BAT remain unclear. Recent studies have shown that glycogen promotes BAT differentiation with glycogen levels decreasing as lipid droplets (LD) size increases during embryogenesis [6]. Glycogen synthase 1 (GYS1) knockout in mouse embryos strongly reduced LD levels in BAT, suggesting a close relationship between glycogen and LD formation. GYS1 knockdown in human preadipocytes yielded similar results.

The importance of STBD1-mediated glycophagy in BAT differentiation is now recognized. Glycophagy markers STBD1 and GABARAPL1 are both expressed in BAT. Moreover, autophagy inhibitors (e.g., chloroquine and wortmannin) and STBD1 knockdown [61] blocked LD biosynthesis in BAT. The promotion of LD formation in BAT is at least partly induced by STBD1-mediated glycophagy glycogenolysis. Interestingly, although glycogen and glycophagy were transiently present in WAT, GYS1 knockdown did not affect LD formation there. Moreover, LD and glycogen clusters are spatially separated [6]. Thus, the mechanism of LD biosynthesis differs between WAT and BAT, a topic that requires further investigation.

4.2 STBD1-dependent glycogen accumulation supportes myoblasts survival during ER stress

Myoblasts are undifferentiated cells implicated in skeletal muscle development and repair of muscle injury during embryonic growth. During myoblasts differentiation, the ER is prone to dysregulation of homeostasis,

inducing ER stress and culminating in the unfolded protein response (UPR), an evolutionarily conserved mechanism of cell survival [62]. Genes encoding enzymes related to glucose and glycogen metabolic processes are associated with the UPR pathway [63]. However, little is understood regarding the relationship between ER stress and glycogen metabolism. We do know that STBD1 is a glycoprotein-binding and ER-resident protein [4]. Under ER stress, STBD1 is strongly upregulated through the PERK signaling pathway downstream of the UPR, inducing STBD1-dependent glycogen accumulation and structure formation to provide energy for myoblast survival [64]. In the absence of ER stress, STBD1 overexpression promotes glycogen accumulation but does not stimulate glycogenesis. These patterns suggest that STBD1 mediates the accumulation of existing intracellular glycogen in the ER without stimulating an increase in glycogen content. Indeed, STBD1 interacts with many glycogen-binding proteins [18]. During ER stress, STBD1, glycogenin (GN) and GS1 co-exist, suggesting that STBD1 acts as a scaffold to mediate GS1 and GN recruitment to the ER, thus promoting glycogen accumulation and protein complex assembly there. Lytridou et al., discovered that STBD1-dependent glycogen accumulation inhibits apoptosis during ER stress, benefiting myoblasts survival. In contrast, STBD1 silencing eliminated glycogen cluster formation and significantly increased apoptosis. These results indicate an essential correlation between glycogen cluster formation and cell survival during ER stress [7]. STBD1 is localized at ER-mitochondrial contact sites, the primary site of autophagosome origin [65]. Therefore, glycogen clusters from ER stress-induced STBD1 activity may be degraded in autophagosomes. However, Lytridou et al. showed that STBD1-dependent glycogen accumulation did not co-localize with Lamp1. Additionally, autophagy inhibitor bafilomycin A1 (BafA1) had no effect on glycogen accumulation, indicating that the ER stress-induced degradation of STBD1-dependent glycogen accumulation differs from classical autophagy and may be a novel STBD1 function [66].

In brief summary, ER stress-induced glycogen synthesis and STBD1-dependent accumulation might be an integral part of myoblast formation, ensuring intracellular glucose supply to support cell differentiation and survival. Further research on these STBD1-dependent processes should shed light on ER homeostatic function and cellular metabolism.

5. STBD1 and non-neoplastic diseases

5.1 STBD1 therapy for Pompe diseases

Pompe disease also known as glycogen storage disorder type II and is a rare autosomal recessive neuromuscular disorder [8]. Pompe disease is primarily attributed to the lack of lysosomal GAA, leading to lysosomal glycogen accumulation and lysosomes enlargement [67], and even severe cardiac and skeletal myopathy [68]. Although, enzyme replacement therapy (ERT) with recombinant human GAA (rhGAA) has potential as an effective treatment for Pompe disease, the technique suffers from numerous limitations that lead to unsatisfactory clinical results. These include the high cost of lifelong high-dose administration, development of high anti-rhGAA antibody concentrations in most patients, and reduced skeletal muscle efficacy [69,70]. Therefore, a new treatment for Pompe disease is essential. Recent studies have revealed that the prevention of cytoplasmic glycogen trafficking into lysosomes could be a feasible approach. STBD1 is a key candidate because it participates in glycogen metabolism and facilitates intracellular glycogen transport to lysosomes [4]. Thus, interfering with STBD1 blocks glycogen transport to lysosomes and inhibits glycophagy, alleviating the symptoms of Pompe disease.

STBD1 expression is specifically enhanced in the skeletal muscles of Pompe disease mice [16]. In GAA-KO mice, the AAV2/9 vector expressing STBD1-specific shRNA blocked STBD1 expression but did not alter lysosomal glycogen accumulation. This result has two possible interpretations: either STBD1 does not participate in lysosomal glycogen transport in skeletal muscles or AAV-shRNA-mediated gene silencing is insufficient to alleviate the transport function of STBD1. To distinguish between these two cases, Sun et al. generated STBD1/GAA double knockout (dKO) mice and investigated the role of STBD1 in glycogen transport to various tissues. Consistent with previous reports, both skeletal and cardiac muscle glycogen accumulation were unaffected in dKO mice. However, dKO mouse liver had 73% lower lysosomal glycogen storage than GAA KO mice [4]. Exogenous expression of human STBD1 in dKO mice then caused liver lysosomal glycogen content to recovery rapidly, suggesting that STBD1 is a major mediator of glycogen

transport into hepatic lysosomes [4]. Autophagy clearly plays a critical role in glycogen in skeletal muscle transport to the lysosomes, as suppressing autophagy reduces lysosomal glycogen content by 60% in the skeletal muscles of GAA KO mice [71]. Although multiple pathways probably exist in the same tissue, each tissue likely has a distinct major pathway for transporting glycogen to lysosomes. Thus, STBD1-mediated glycophagy is prominent in glycogen transport to hepatic lysosomes but is not majorly involved in the cardiac and skeletal muscle. Moreover, the mechanism of glycogen transport to lysosomes is likely different between liver and muscle, suggesting that treatment regimens for Pompe disease may need to be adjusted according to tissue type. Overall, available findings indicate that interfering with STBD1 function may be a viable therapeutic option for excess glycogen accumulation in the liver. As STBD1 is also highly expressed in the cardiac and skeletal muscles, a major breakthrough for curing Pompe disease may arise with further investigation of STBD1's secondary transport mechanism in these tissues.

5.2 STBD1-mediated glycophagy dysfunction aggravates diabetic cardiomyopathy

Diabetes mellitus (DM) is a metabolic disease with both genetic and environmental causes. Cardiovascular complications are the leading cause of death in patients with DM [72], and diabetic cardiomyopathy (DCM) has attracted increasing attention [73]. This complication is characterized by cardiac insufficiency without coronary heart disease, hypertension, valvular disease, congenital heart disease, or any other cardiovascular disorders. Pathological glycogen deposition in the heart was evident among diabetic patients with DCM and among experimental models [74-76]. Some studies have shown that glycophagy exists in the heart and is responsive to abnormal glycemic stimuli in vivo [10]. STBD1-mediated glycophagy may be a pivotal link between diabetes and glycogen mishandling in the heart [77].

Previous studies have shown that glycogen in hepatocytes and skeletal muscle cells is continuously depleted during fasting, whereas cardiac glycogen remains unchanged or even accumulates [78-80]. This probably occurs because glucose is redirected to critical tissues (e.g., heart and brain) for maintaining essential organ function under nutritional deficiency [81]. In patients with diabetes, disrupted glucose metabolism and impaired insulin signaling pathways underlie cardiac glycogen pathology [82]. Mellor et al. found that, under insulin and high glucose conditions, the glycophagy marker proteins STBD1 and GABARAPL1 were significantly elevated in neonatal rat ventricular myocytes (NRVMs). The results from streptozotocin-treated diabetic rat's hearts also supported this finding, suggesting diabetes induced STBD1-mediated glycophagy in cardiocytes. This study first demonstrated that glycogen pathology in DCM is associated with glycophagy induction. Interestingly, women with diabetes are at higher risk of heart failure than men, the mechanisms of sex-dependent metabolic stress-related cardiac pathology have not been elucidated. Reichelt et al. found that STBD1 expression was increased in the female heart compared with male heart in cardiac metabolic stress settings [10,83]. This sex difference may result in different predispositions to DCM among patients [10], and further confirms the disease's strong association with STBD1-mediated glycophagy.

The PI3K/Akt/mTOR [84] and the AMPK [85] pathways are well-known regulators of autophagy, and play essential roles in DCM. In NRVMs, extracellular insulin concentrations increase STBD1 expression, which correlates with the insulin-regulated PI3K/Akt signaling pathway [86]. In addition, fasting-induced STBD1 and GABARAPL1 upregulation is also associated with in vivo Akt activation [10]. FoxO1 and FoxO3, which are inhibited by Akt activation, bind directly to the GABARAPL1 promoter in the nucleus and regulate its transcription [52]. Therefore, the Akt pathway may inhibit glycophagy and aggravate DCM through repressing FoxO-mediated GABARAPL1 transcription. Recently, a chemical genetic screen revealed that STBD1 is a substrate of AMPK and may also regulate DCM through the AMPK pathway [87]. Taken together, the comprehensive regulation of STBD1-mediated glycophagy through PI3K/Akt and AMPK signaling pathways may more clearly illustrate the disease mechanism and provide a direction for DCM treatment. (**Figure 4**)

5.3STBD1-mediated glycophagy activation alleviates ischemic myocardial injury

Myocardial ischemia leads to disturbances in myocardial metabolism [88]. In the cytosol, cytosolic phosphorylases and debranching enzymes are the main regulators of glycogen degradation, whereas glycophagy

isolates glycogen into autophagosomes for degradation in lysosomes [42]. Glycophagy thus provides additional metabolic support for the ischemic stress response [89]. STBD1-mediated glycophagy optimizes cytoplasmic energy metabolism, providing valuable applications to the prevention and treatment of myocardial ischemia.

A recent study found that Asiatic acid (AA) protects against ischemic myocardial injury by regulating energy metabolism through STBD1-mediated glycophagy [90]. Long used in wound therapy against focal cerebral ischemia and hepatic ischemia/reperfusion injury, AA mitigates oxidative stress and maintains mitochondrial homeostasis. Thus its therapeutic effect probably lies in the regulation of metabolic homeostasis [11,91]. Studies have demonstrated that AA elevates glycophagy markers STBD1 and GABARAPL1 and promotes their interaction, indicating that AA treatment upregulates glycophagy in cardiomyocytes. Moreover, STBD1 and GABARAPL1 expression and interaction were associated with activation of the PI3K/Akt axis [10,86,92]. Treatment with PI3K or Akt inhibitors blocked the stimulatory effect of AA on glycophagy. These results indicated that AA protects against ischemic myocardial injury via regulating STBD1-mediated glycophagy through the PI3K/Akt pathway (**Figure 4**).

Interestingly, glycogen-containing lysosomes maintain glycogen storage through autophagy, implying that glycogen accumulation and glycogenolysis co-exist in autolysosomes [93]. Thus, either glycogen storage or glycophagy protects against myocardial ischemia or energy stress. Glycogen storage in autolysosomes may be a defense mechanism in response to severe cardiac ischemia, but excessive accumulation during energy stress is harmful to the body [94]. Hence, STBD1-mediated glycophagy is indispensable for glycogen degradation to protect against ischemic myocardial injury. If impaired, the resultant abnormal glycogen metabolism poses a major health risk.

5.4STBD1 is a potential risk locus for Parkinson's disease

Parkinson's disease (PD) is a neurodegenerative disorder characterized by early death of dopaminergic neurons in the substantia nigra pars compacta. The resultant dopamine deficiency within the basal ganglia leads to classical parkinsonian motor symptoms [95], including bradykinesia, muscular rigidity, rest tremor, as well as postural and gait impairment [96]. While originally thought to be caused primarily by environmental factors, PD is now known to have a genetic component [97,98]. Notably, STBD1 is predicted to be a risk factor for PD. A genome-wide association (GWA) meta-analysis of 12,386 PD cases and 21,026 controls discovered and confirmed 11 relevant loci [9]. After analyzing 1,920 SNPs, five additional PD risk loci (PARK16/1q32, STX1B/16p11, FGF20/8p22, STBD1/4q21, and GPNMB/7p15) were identified. Two (PARK16/1q32 and FGF20/8p22) of these five have been mentioned in previous association studies [99,100]. Furthermore, analysis performed on a dataset of post-mortem brain samples revealed that methylation and gene expression changes in PARK16/1q32, GPNMB/7p15, and STX1B/16p11 loci were associated with PD risk variants [9]. These findings provide insight into molecular mechanisms and candidate genes affecting PD risk, including*STBD1* . Additional association signals for STBD1, LRRK2, and SPATA19 in PD have also been recently uncovered [101]. These studies all indicated that STBD1 is a possible risk locus that can trigger PD pathogenesis.

6. STBD1 suppresses cancer pathogenesis

Over the years, available research has uncovered the role of autophagy dysregulation in a broad range of diseases, particularly cancer. The involvement of autophagy in tumor pathogenesis is well-established, and activation of autophagy enables cancer cells to survive even under stressful conditions [102]. Suppressing autophagy leads to tumorigenesis because genotoxic cellular waste accumulates, facilitating additional genomic mutations [103,104]. These two contrasting effects suggest that autophagy is a double-edged sword in cancer pathogenesis. Collectively, genomic, transcriptomic, and proteomic analyses of human cancer samples support the conclusion that autophagy has dual effects [105,106]. Thus, when faced with tumors, the body appears to repeatedly regulate autophagy-associated genes (ATGs) and autophagy regulating factors involved in cancer pathogenesis. However, we currently have few studies on whether such alterations to ATGs generate changes in selective autophagy (i.e., excessive activation or selective autophagy dysfunction), nor do we understand the exact relationship between those changes and cancer.

To explore the relationship between aberrant selective autophagy and cancer, Han et al. developed "inference of cancer-associated LIR-containing proteins" (iCAL). This pipeline integrated a new algorithm named "prediction of the LIR motif" (pLIRm) to predict LIR motif-associated mutations (LAMs) [12]. Using iCAL, LIR-containing proteins (LIRCPs) that carry single point mutations in the LIR motif were identified, including well-established *ATG* s and autophagy regulators, and many novel candidate genes. Five of these proteins (STBD1, ATG4B, EHMT2, BRAF, and ERCC6) were selected for experimental validation, and all except ERCC6 exhibited changes to both LC3 binding and autophagy [12].

Although the connection between autophagy and cancer is well established, the role of glycophagy and therefore STBD1 in cancer development remain unclear. The Gene Set Cancer Analysis (GSCA) (http://bioinfo.life.hust.edu.cn/GSCA/#/) shows that STBD1 expression was downregulated in multiple tumors, including breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), head and neck squamous cell carcinoma (HNSC), lung squamous cell carcinoma (LUSC), bladder urothelial carcinoma (BLCA) and stomach adenocarcinoma (STAD). Of these, STBD1 expression was lowest in BRCA (**Figure 5A**). Notably, analysis of an intestinal adenocarcinoma sample revealed a type II mutant (W203C) in the STBD1 LIR sequence, which interacts with GABARAPL1 [107]. W203C mutation was similar to STBD1 knockdown in vitro and in vivo, implying that STBD1 inhibits tumor growth through its interaction with GABARAPL1. Moreover, immunofluorescence experiments and glycogen content determination demonstrated that overexpression of STBD1 W203C, but not wild-type STBD1, abolished the co-localization with GABARAPL1 and caused glycogen accumulation in HCT116 cancer cells.

To further clarify how STBD1 inhibits tumor growth, a study compared gene expression profiles of shControl (control shRNA) and shSTBD1(shRNA targeting STBD1) in HCT116 cells. Researchers first generated shSTBD1 HCT116 cells overexpressing with shRNA-resistant wild-type STBD1 (shSTBD1/WT) and STBD1W203C (shSTBD1/W203C). Overexpression of shSTBD1/WT, but not STBD1/W203C, blocked tumor growth in a xenograft model. Similarly, RNAseq, quantitative RT-PCR, and bioinformatics showed that STBD1 deletion significantly enhanced cancer-typical characteristics, including persistent proliferation, genomic instability, cell death, invasion, metastasis, and heightened metabolism [108]. Mechanistically, STBD1 affected cancer-related pathways. STBD1 depletion upregulates AKT1 and inhibits tumor suppressor NFKB1, indicating that STBD1 acts through the AKT1/NFKB1 pathway to influence cancer development [109]. Other mechanisms, such as the glycolysis-gluconeogenesis pathway, have been identified. Consistent with its role as a glycogen transporter, STBD1 knockout increased the expression of various enzymes and intermediate products involved in glycolysis. Moreover, STBD1 W203C induces changes in gene expression patterns are similar to STBD1 silencing [12]. Bioinformatics analysis (http://bioinfo.life.hust.edu.cn/GSCA/#/) predicted that STBD1 inhibited pan-cancer through the apoptosis, cell cycle, androgen receptor, and DNA damage response pathways, whereas the protein activated cancer through the PI3K/AKT, TSC/mTOR, RAS/MAPK, RTK, and estrogen receptor pathways (**Figure 5B**). Therefore, STBD1 suppresses tumor growth through multiple mechanisms.

To confirm these results, a meta-analysis of shControl and shSTBD1 in HCT116 cells was performed. STBD1 inhibition activates the pentose phosphate pathway, which in turn enhances glycolysis promotes the TCA cycle and boosts nucleotide biosynthesis [12].Notably, the metabolic assay results were largely consistent with transcriptome data, including the upregulation of multiple enzymes and intermediate products critical to the glycolytic pathway. The known glycolysis inhibitor 2-deoxyglucose (2-DG) was found to inhibit the proliferation of STBD1-suppressed cells more than that of control cells[110]. Thus, using glycolysis inhibition could be a beneficial therapeutic option for cancer patients with low STBD1 expression or mutant *STBD1* .

To summarize, iCAL allowed for detailed analysis of the interrelationship between cancer and abnormal selective autophagy (e.g., STBD1-mediated glycophagy). STBD1 overexpression suppresses cancer pathogenesis, clarifing the missing link between glycolysis and tumor suppression. Through ICAL , untapped autophagic pathways could be exploited to identify novel cancer therapies (e.g., developing novel agonists or drugs to specifically promote STBD1 expression) Treatments targeting these autophagic pathways, including STBD1-mediated glycophagy, will hopefully improve survival and quality of life in cancer patients [17].

7. Conclusion and future prospects

In this review, we summarized STBD1 molecular structure, subcellular localization, and tissue distribution and expounded that STBD1 as a glycogen cargo receptor involved in glycophagy. We highlighted the protein's biological functions and its significance in myocardial diseases, central nervous system diseases, and neoplastic diseases. Our conclusion is that STBD1 is a promising therapeutic target for these diseases.

STBD1 possesses two well-defined structural motifs. Of these, the leucine zipper motif remains poorly understood, although recent studies have demonstrated that the leucine zipper motif mediates the dimerization of P-selectin glycoprotein ligand-1 [111], This link promises to be a new direction in research on STBD1 motifs.

Although this review focused on STBD1 localization to the plasma membrane and ER, the protein is also found in the Golgi apparatus, mitochondria, and peroxisomes. Thus, STBD1 may act as a novel resident protein for other subcellular organelles with untapped biological functions. Notably, STBD1 is also localized to the MAM, playing a novel role in glucose homeostasis [23]. STBD1 knockout increased ER-mitochondria association and enhanced mitochondrial fragmentation in the liver. The distortions in the integrity of hepatic MAM are closely related to insulin resistance [112,113]. Insulin resistance is a common metabolic abnormality in type 2 diabetes (T2D) [114]. Thus, it is important to explore the molecular mechanism between STBD1 and insulin resistance for T2D treatment in the near future.

In terms of STBD1 involvement in tumors, the ICAL pipeline revealed that STBD1 knockdown or disruption of its binding to LC3 enhanced glycolysis and upregulated the pentose phosphate pathways in cancer cells, thereby promoting tumor growth. Notably, glycolysis inhibitor 2-DG blocked the growth tumor tissue that lowly expressed STBD1 [110]. Therefore, targeting the glycolysis and pentose phosphate pathways could be a novel therapeutic strategy for cancer patients with low STBD1 expression and STBD1 mutations. Interestingly, GSCA predicted a correlation between*STBD1* expression and drug sensitivity from the Genomics of Therapeutics Response Portal (CTRP). Five drugs were hypersensitive to STBD1 expression, including GSK-J4 [115], LY-2183240 [116], doxorubicin [117], topotecan [118], and belinostat [119]. All of these drugs act on genetic material. Further research is needed to determine whether these drugs can affect *STBD1* gene transcription and regulate protein a activity, thus suppressing tumors. Regardless, STBD1 has enormous potential as a therapeutic target, and its comprehensive clinical application will greatly benefit the development of cures for many diseases.

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Figure legends:

Figure 1 . Overview of STBD1 structure and gene sequences

(**A**) The different structural domains of STBD1. (**B**) The topology of STBD1 in the membrane. (**C**) Comparison of amino acid sequences of STBD1 proteins from different species, including mouse, rat, and human. The ESPript software is used to analyze the conservativeness of STBD1 amino acid sequences among different species. Amino acid sequences marked with red background are mean consistent. The amino acid sequences marked with blue rectangles are mean similar. STBD1, Starch-binding domain-containing protein 1.

Figure 2 . The subcellular localizations and tissue distributions of STBD1

(**A**) The subcellular locations of STBD1 from the COMPARTMENTS database. STBD1 is located in the ER, plasma membrane, cytosol, lysosomes, extracellular space, Golgi apparatus, endosomes, nuclei, peroxisomes, and mitochondria. (**B**). The tissue expressions of STBD1 mRNA in the Human Protein Atlas. STBD1 mRNA is distributed in the skeletal muscle, tongue, liver, adipose tissue, and heart muscle. STBD1, Starch-binding domain-containing protein 1.

Figure 3 . Relevant signal pathways mediatingSTBD1-dependent glycophagy .

STBD1 interacts with GABARAPL1 to transport glycogen to the autophagosome, where glycogen is degraded by GAA. Glycophagy is mainly activated through the cAMP/ PKA, G6PC/SIRT1/ FoxO and Ca^{2+} signaling pathways. Its primary inhibitory pathways are PI3K/mTOR and Akt/FoxO signaling pathways. GAA, acid α-glucosidase; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; SIRT1, silencing information regulatory factor 2 related enzymes 1; FoxO, forkhead box-containing protein O; PI3K, phosphatidylinositol 3-kinase; PP2A, protein phosphatase 2A; TSC 2, tuberous sclerosis complex 2; PPARα, proliferator-activated receptor α· GABARAPL1, γ-aminobutyric acid receptor-associated protein-like 1;G6P, glucose-6-phosphate; G6PC, glucose-6-phosphatase; AMPK, AMP-activated protein kinase; Akt, protein kinase B; mTOR: mammalian target of rapamycin; STBD1, Starch-binding domain-containing protein 1.

Figure 4 . The mechanisms of STBD1-mediated glycophagy in cardiomyocytes .

On the one hand, insulin activates the PI3K/Akt signaling pathway, suppressing FoxO1 expression and GABARAPL1 transcription. These pathways aggravate diabetic cardiomyopathy by inhibiting STBD1-mediated glycophagy. On the other hand, AA facilitates STBD1-mediated glycophagy through the PI3K/Akt signaling pathway and regulates intracellular energy homeostasis, alleviating ischemic myocardial injury. AA, Asiatic acid; PI3K: phosphatidylinositol 3-kinase; Akt, protein kinase B; FoxO, forkhead box-containing protein O; GABARAPL1, γ-aminobutyric acid receptor-associated protein-like 1; STBD1, Starch-binding domain-containing protein 1.

Figure 5.

Bioinformatics analysis of STBD1 in pan-cancer

(A) STBD1 is downregulated in multiple cancers. STBD1 is downregulated in BRCA, COAD, HNSC, LUSC, BLCA and STAD. Data derived from the GSCA database. (FDR<0.05) (B) The percentage of cancers in which STBD1 's mRNA expressions have potential effect on pathway activity (FDR<0.05). BRCA, breast invasive carcinoma; COAD, colon adenocarcinoma; HNSC, head and neck squamous cell carcinoma; LUSC, lung squamous cell carcinoma; BLCA, bladder urothelial carcinoma; STAD, stomach adenocarcinoma; STBD1, Starch-binding domain-containing protein 1.

Fig 1.

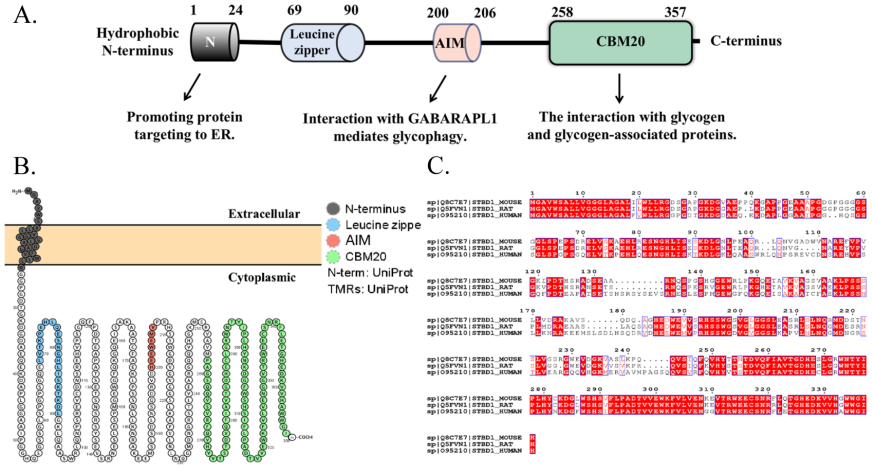
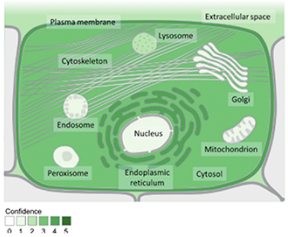


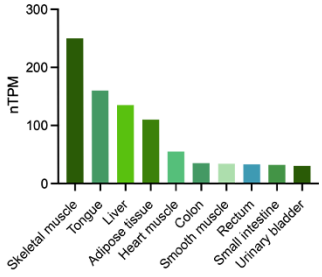
Fig 2.

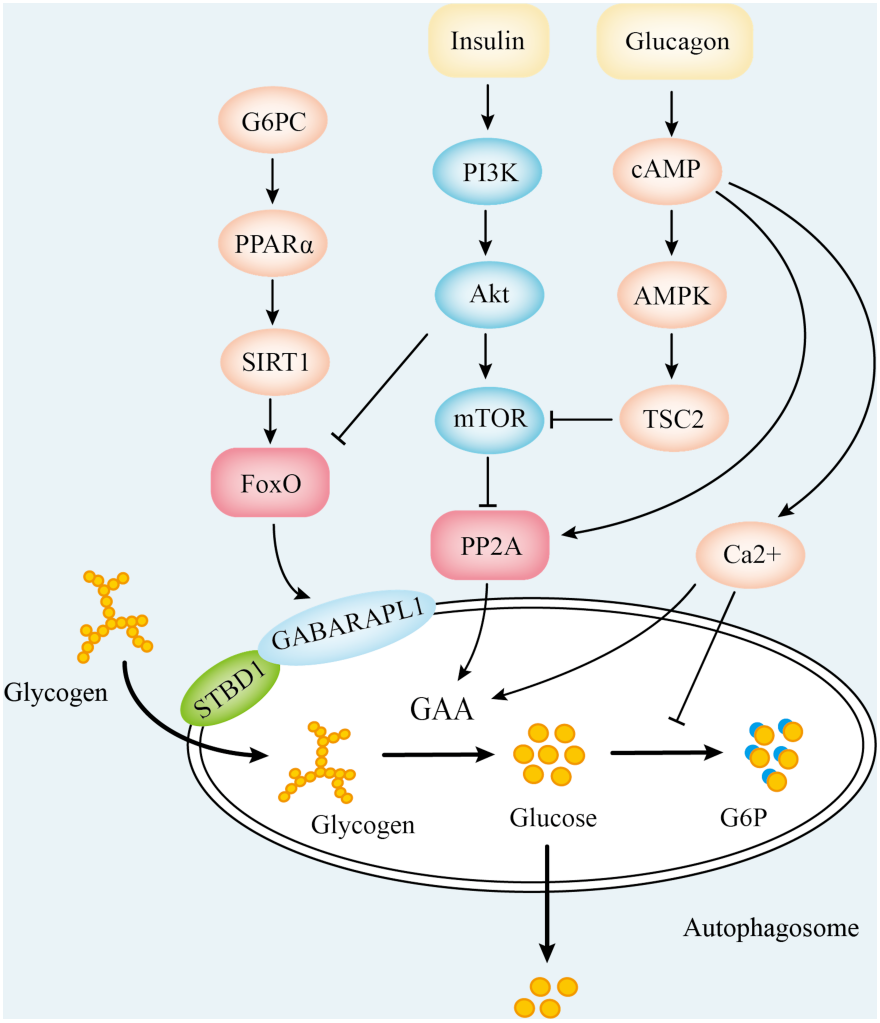
A.



Compartment	Confidence
endoplasmic reticulum	4
plasma membrane	4
cytosol	3
lysosome	2
extracellular	2
golgi apparatus	1
endosome	1
nucleus	1
peroxisome	1
mitochondrion	1
cytoskeleton	1

B.





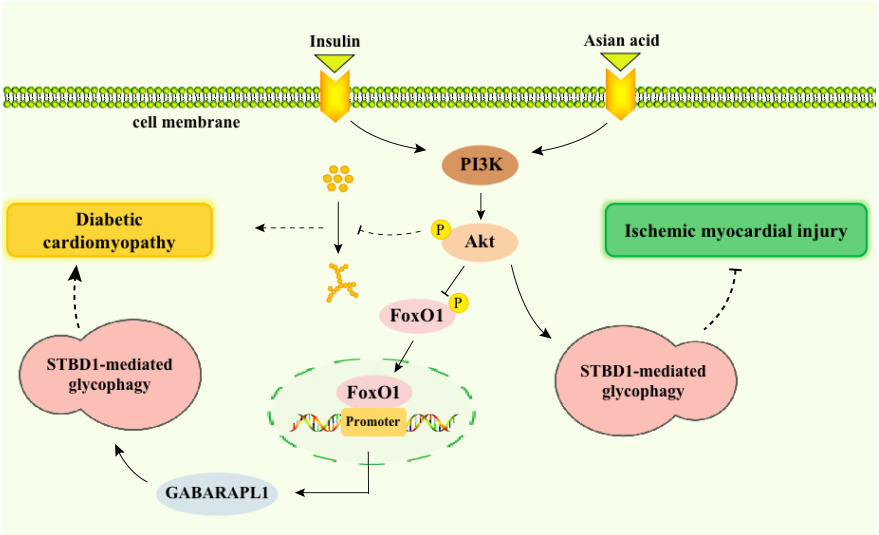
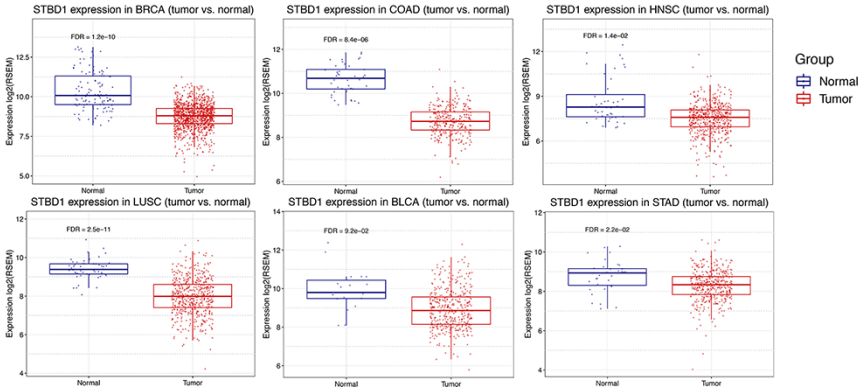


Fig 5.

A.



B.