

Glycogen synthesis and beyond, a comprehensive review of GSK3 as a key regulator of metabolic pathways and a therapeutic target for treating metabolic diseases

Li Wang^{1,2,3,4,5} | Jiajia Li^{2,3,4,5} | Li-jun Di^{2,3,4,5} 

¹Proteomics, Metabolomics, and Drug Development Core, Faculty of Health Sciences, University of Macau, Macau, China

²Department of Biomedical Sciences, Faculty of Health Sciences, Macau, China

³Cancer Center of the Faculty of Health Sciences, University of Macau, Macau, China

⁴Institute of Translational Medicine, University of Macau, Macau, China

⁵Ministry of Education, Frontiers Science Center for Precision Oncology, University of Macau, Macau, China

Correspondence

Li-jun Di and Li Wang, Faculty of Health Sciences, University of Macau, Macau SAR, China.

Email: lijundi@um.edu.mo and liwang@um.edu.mo

Funding information

Science and Technology Development Fund (FDCT) of Macao SAR,

Grant/Award Numbers: FDCT/0014/2018/A1, FDCT/0117/2018/A3; National Natural Science Foundation of China,

Grant/Award Number: NSFC 81772980;

Universidade de Macau,

Grant/Award Number:

MYRG2018-00158-FHS

Abstract

Glycogen synthase kinase-3 (GSK3) is a highly evolutionarily conserved serine/threonine protein kinase first identified as an enzyme that regulates glycogen synthase (GS) in response to insulin stimulation, which involves GSK3 regulation of glucose metabolism and energy homeostasis. Both isoforms of GSK3, GSK3 α , and GSK3 β , have been implicated in many biological and pathophysiological processes. The various functions of GSK3 are indicated by its widespread distribution in multiple cell types and tissues. The studies of GSK3 activity using animal models and the observed effects of GSK3-specific inhibitors provide more insights into the roles of GSK3 in regulating energy metabolism and homeostasis. The cross-talk between GSK3 and some important energy regulators and sensors and the regulation of GSK3 in mitochondrial activity and component function further highlight the molecular mechanisms in which GSK3 is involved to regulate the metabolic activity, beyond its classical regulatory effect on GS. In this review, we summarize the specific roles of GSK3 in energy metabolism regulation in tissues that are tightly associated with energy metabolism and the functions of GSK3 in the

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2021 The Authors. *Medicinal Research Reviews* published by Wiley Periodicals LLC.

development of metabolic disorders. We also address the impacts of GSK3 on the regulation of mitochondrial function, activity and associated metabolic regulation. The application of GSK3 inhibitors in clinical tests will be highlighted too. Interactions between GSK3 and important energy regulators and GSK3-mediated responses to different stresses that are related to metabolism are described to provide a brief overview of previously less-appreciated biological functions of GSK3 in energy metabolism and associated diseases through its regulation of GS and other functions.

KEYWORDS

energy homeostasis, glucose metabolism, GSK3, insulin sensitivity, metabolic regulators, stress response

1 | INTRODUCTION

Glycogen synthase kinase-3 (GSK3), a highly evolutionarily conserved intracellular serine/threonine kinase, was originally found to inhibit glycogen synthase (GS) via phosphorylation and to modulate glucose metabolism.¹ GSK3 has important regulatory functions in glucose metabolism, insulin activity, and energy homeostasis. In response to insulin, GSK3 is phosphorylated and inactivated, which allows GS activation and glucose deposition as glycogen. Acute or chronic treatment of animal models with obesity and/or type 2 diabetes (T2D) by small cell-permeable inhibitors of GSK3 stimulates GS activity, glucose metabolism, and insulin action in skeletal muscle and liver,²⁻⁵ leading to improved insulin sensitivity and glucose homeostasis.^{2,6-9} In contrast, GSK3 negatively affects insulin signaling by phosphorylating IRS1 and promotes its degradation in the liver and muscle.¹⁰⁻¹⁴

Subsequently, GSK3 has been confirmed to be among the protein kinases with the most substrates in the cell.^{15,16} More than 40 proteins are regulated by GSK3, which influences various cellular processes.¹⁵ In addition, multiple cell signaling pathways have been demonstrated to be involved with GSK3 signaling, such as the PI3K/Akt, Wnt, Hedgehog, cyclic adenosine monophosphate (cAMP), MAPK, and transforming growth factor-beta (TGF- β) signaling pathways.^{17,18} Therefore, GSK3 is associated with a wide range of cellular processes, such as cell proliferation, differentiation, apoptosis, the cell cycle, the immune response, and organ development.^{15,19-21} Consequently, GSK3 is implicated in multiple diseases, such as cancer, neurodegenerative diseases, such as Alzheimer's, bipolar disorder (BPD), psychiatric disorders, Parkinson's, aging, inflammatory conditions, and metabolic disorders, including diabetes, cardiovascular disease, and atherosclerosis.²²⁻²⁷ GSK3 activity has recently been addressed again in insulin resistance, obesity, neurodegenerative diseases, stress responses, inflammation, and energy metabolism.

Mammalian GSK3 exists as two isoforms, GSK3 α (51 kDa) and GSK3 β (47 kDa).^{12,17,28} These two isoforms are encoded by two distinct genes located on chromosomes 19 and 3, respectively. But they share 97% amino acid sequence similarity in their catalytic domains and 84% amino acid sequence similarity overall (Figure 1A).^{29,30} Therefore, these two isoforms have some common biological functions.³¹⁻³³ GSK3 was traditionally considered a cytosolic protein, but it was also found in other cell compartments in addition to the cytosol, such as the nucleus and mitochondria, as verified by both microscopy and immunoblot experiments.³⁴⁻³⁷ In contrast to the numerous

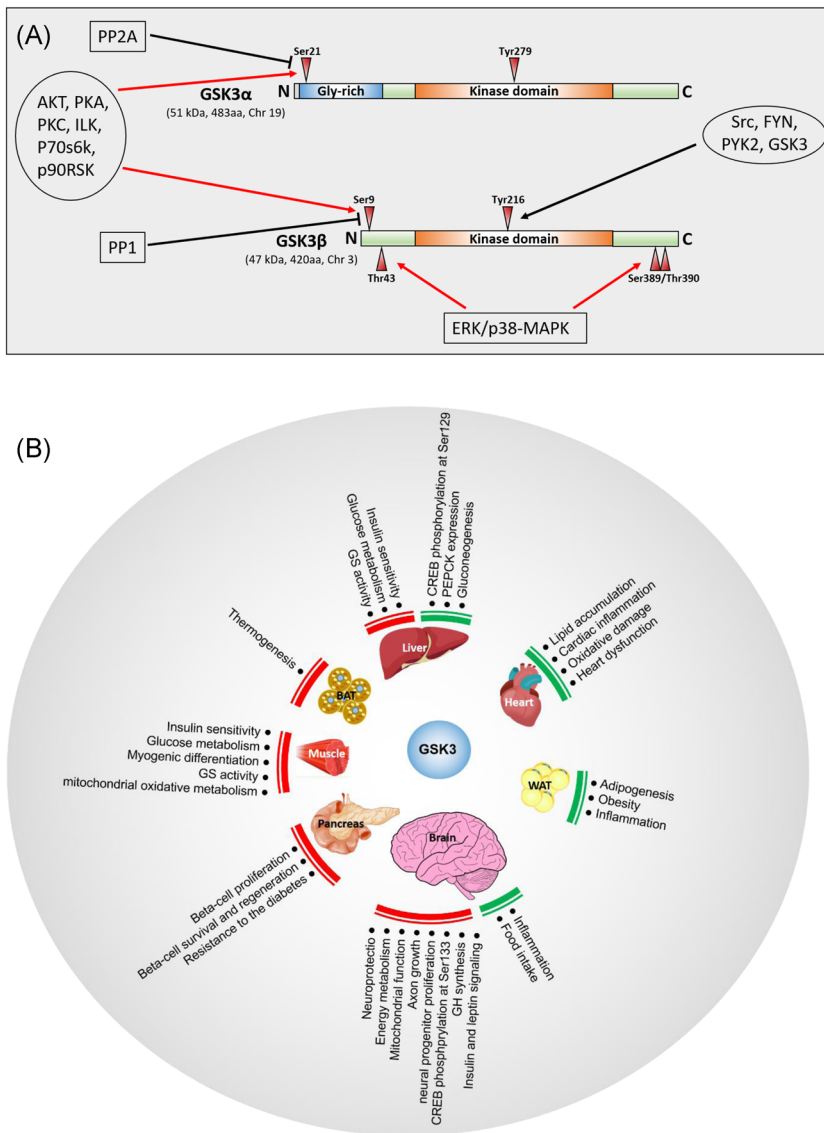


FIGURE 1 GSK3 mediates metabolic phenotypes in various tissues. (A) Comparison of the structure, functional domains, and phosphorylation sites of the two GSK3 isoforms. Some phosphatases acting on GSK3 are also indicated. (B) Main metabolic tissues, such as liver, heart, brain, white adipose tissue (WAT), brown adipose tissue (BAT), and skeletal muscle are depicted to illustrate GSK3 functions. In the liver and the brain, GSK3 has both positive (green) and negative (red) regulatory roles targeting different downstream pathways. In the liver and the brain, GSK3 mainly serves as the negative regulatory factor of beneficial effects, while in the heart and WAT, GSK3 mainly acts as the positive regulatory factor of harmful effects. By serving as the negative regulator, GSK3 inhibits the GS activity, insulin and glucose metabolism pathways in the liver, skeletal muscle, and the brain. Particularly, GSK3 also inhibits the thermogenesis in BAT, β -cell proliferation, survival and regeneration in the pancreas and energy metabolism in the brain. By serving as the positive regulator, GSK3 promotes the CREB activity in the liver, lipid accumulation, cardiac inflammation and oxidative damage in the heart, adipogenesis, obesity and inflammation in WAT, etc. GSK3, glycogen synthase kinase-3 [Color figure can be viewed at wileyonlinelibrary.com]

activities and effects of GSK3 β in the cytosol and nucleus, the potential role of GSK3 β in mitochondria remains to be discovered. GSK3 α has an extended glycine-rich N-terminal region that excludes it from the nucleus and distinguishes its biological function from that of GSK3 β . Deletion of this region or calcium stimulation induces the nuclear accumulation of GSK3 α .³⁰

Despite the high degree of homology, GSK3 β function cannot be compensated by GSK3 α , as shown by GSK3 β knockout in mice being embryonically lethal, while GSK3 α knockout animals are viable (with the deletion of exon 2). GSK3 α knockout mice showed enhanced glucose and insulin sensitivity, reduced fat mass, and fewer abnormalities in neuronal development.^{12,38–40} Despite the metabolic benefits, the global deletion of GSK3 α in mice was reported to cause cardiac hypertrophy, contractile dysfunction, early mortality, and other age-related pathologies in multiple tissues. These pathological phenotypes were found to be associated with activated mTORC1 and suppressed autophagy.^{41,42} An essential role of GSK3 β in regulating cardiac homeostasis in response to stresses was also well-defined by several *in vitro* and *in vivo* studies,⁴³ suggesting more attention is deserved when targeting GSK3 for relevant disease treatment (discussed in detail in cardiac tissue section of this article).

Interestingly, there are two conflicting phenotypes observed on male fertility associated with the deletion of exon 2 of GSK3 α . MacAulay et al.¹² reported that the global knockout of GSK3 α exon 2 displays normal fertility. However, Maurin et al. observed that male mice lacking exon 2 of GSK3 α are infertile. Incomplete Cre-mediated deletion of GSK3 α may occur to the germline cells which causes the fertile phenotype.⁴⁴ Further study demonstrated that deletion of exon 2, 3, and 4 of GSK3 α leads to male infertility partially owing to the impaired glucose utilization and the lower sperm ATP levels.^{45,46}

GSK3 activity is dynamically regulated by several mechanisms. The best-known regulation is the inhibitory phosphorylation of GSK3 at an N-terminal Ser residue (at Ser21 of GSK3 α or Ser9 of GSK3 β) through either well-defined PI3K/AKT pathway or other kinases including PKC, protein kinase A (PKA), integrin-linked kinase (ILK), p70S6K, and p90RSK.^{47,48} The phosphorylation of Ser9/21 of GSK3 could be dephosphorylated by protein phosphatase 1 (PP1) and PP2A, respectively.⁴⁹ In addition, Ser9 phosphorylation of GSK3 β could be facilitated by the phosphorylation at Thr43 and Ser389/Thr390 residues via ERK- and p38-MAPK (Figure 1A).^{50,51} GSK3 activity can also be stimulated by phosphorylation at Tyr residue (at Tyr279 of GSK3 α or Tyr216 of GSK3 β). While the mechanisms that regulating Tyr phosphorylation of GSK3 are not well-defined, some kinases such as Src, Fyn, and PYK2 (mainly acting on Tyr216) might be responsible for Tyr279/216 phosphorylation (Figure 1A).⁴⁸ Moreover, the Tyr279/216 phosphorylation could be the consequence of the autophosphorylation.⁵² GSK3 also shows distinct activity according to its subcellular localization (i.e., cytosol, nucleus or mitochondria). Another potential regulatory mechanism of GSK3 activity is the phosphorylation status of its substrates. Many GSK3 substrates require to be primed before being targeted by GSK3.⁵³ Finally, GSK3 activity could be regulated by the state of pathways containing GSK3. For example, in the canonical Wnt signaling pathway, GSK3 is inactivated upon Wnt signaling activation.

The widespread biological and pathophysiological functions of GSK3 are also associated with the ubiquitous expression of GSK3 in many cell types, such as neurons, hepatocytes, myoblasts, and adipocytes, and multiple tissues such as skeletal muscle, liver, pancreas, and adipose tissue. These cells and tissues are associated with the regulation of energy metabolism and homeostasis. Moreover, GSK3 directly modulates mitochondrial activity, mitochondrial biogenesis, and mitochondrial components in different tissues and influences energy metabolism in addition to its classical roles in the regulation of GS. Recent studies have shown that GSK3 activation is induced by stressors such as hypoxia and reactive oxygen species (ROS) and mediates stress-induced cell death, suggesting that GSK3 is a critical determinant of cell fate by modulating metabolic activity in response to stress. In this review, we present an overview of the function of GSK3 in different tissues by focusing on its regulatory role in energy metabolism and exploring the mechanisms associated with the stress response and the involvement of inflammatory pathways in GSK3-associated diseases. In addition, we describe the cross-talk between GSK3 and some important energy sensors and regulators, such as PGC1 α , Sirt1, AMP-activated protein kinase (AMPK), leptin and

adiponectin, and the mutual regulation among them, which provides evidence for GSK3 acting as an energy metabolism regulator in addition to its regulation of other cellular systems.

1.1 | The tissue-specific function of GSK3 in regulating energy metabolism

Nearly all tissues in the body consume energy. However, not all of them are specialized for regulating energy production, storage, and expenditure like skeletal muscle, adipose tissue, or liver tissue. The availability of tissue-specific GSK3-knockout animal models has facilitated the exploration of the function of GSK3 in regulating energy metabolism in some of these functionally specialized tissues (Figure 1B). These studies revealed that GSK3 is a critical player in many important biological processes by regulating energy metabolism.

2 | GSK3 IN SKELETAL MUSCLE

The expression of GSK3 was originally reported in skeletal muscle, followed by the detection of its ubiquitous expression in multiple cells and tissues. GSK3 β is expressed at a higher level than GSK3 α in both murine and human skeletal muscle.⁵⁴ In addition, inactive phosphorylation of GSK3 α was enhanced when GSK3 β was specifically deleted in skeletal muscle, suggesting that GSK3 α is not able to compensate for the loss of GSK3 β in skeletal muscle.³ Supporting the negative role of GSK3 on insulin action and glucose metabolism, both T2D patients and animal models with insulin resistance showed significantly enhanced GSK3 protein levels and activity in skeletal muscle.^{7,38,55,56}

The mouse models with skeletal muscle-specific GSK3 β knocked out, GSK3 β overexpressed or that carry a mutation at the insulin-responsive phosphorylation site, demonstrated that the negative regulation of GSK3 β on glucose metabolism and insulin sensitivity is mediated by inhibited glycogen deposition and insulin-induced activation of GS in skeletal muscle.^{3,54,57} In contrast, GSK3 α -specific loss in skeletal muscle did not show any improvement in metabolic phenotype,⁵⁸ suggesting that GSK3 β acts as a skeletal muscle-specific GS kinase. Some studies have suggested that GSK3 participates in insulin signaling and glucose metabolism in skeletal muscle by regulating other pathways in addition to regulating GS activity or glycogen synthesis.^{2,59} For instance, transient GSK3 inhibitor treatment (single dose at 30 mg/kg of CHIR98023) caused increased GS activity but no effects on glucose uptake in rat muscles *in vivo*.² Consistently, despite activated GS by acute treatment of these two inhibitors, little acute effect on glucose uptake was observed.⁶⁰ An upregulated insulin receptor substrate-1 (IRS-1) is indicated to augment insulin action on sustained glucose uptake stimulated by chronic treatment of GSK3 inhibitors (CHIR98023 and CHIR 98014 at 2.5 μ M for up to 96 h).⁶⁰ Consistently, GSK3 β overexpression resulted in decreased IRS-1 in mouse skeletal muscle and knockdown of GSK3 α increased IRS-1 level in human skeletal muscle cells,^{10,57} suggesting IRS-1 could be a mediator of GSK3 on insulin action. GLUT4 (glucose transporter 4) plays a dominant role in regulating whole-body glucose homeostasis via transporting glucose across the cell membrane into cells. It is highly expressed in adipose tissue and skeletal muscle.¹² GLUT4 was upregulated in the muscle of ob/ob mice with long-term treatment of GSK3 inhibitor (L803-mts, daily for 3 weeks at 400 nM), suggesting GLUT4 may also participate in GSK3 inhibitor-mediated glucose homeostasis and hyperglycemia prevention.⁹ Importantly, GSK3, particularly GSK3 β , can directly phosphorylate IRS-1 to inhibit the tyrosine phosphorylation activity of insulin receptor,¹⁴ leading to attenuated insulin action. These studies indicate that GSK3 may inhibit insulin action and regulate glucose metabolism not only by repressing GS but also by targeting other cellular factors, thus providing some evidence on the therapeutic benefit of GSK3 inhibition beyond its activation of GS. Importantly, the effects of GSK3 in skeletal muscle seems a long-term consequence collectively from not only GS but also from other GSK3 substrates.

How to reconcile these observations of GS-dependent and GS-independent glucose disposal remains a challenge. However, the differing nature of research approaches, i.e., the genetic approach versus the pharmacological approach, might be part of the reason for discrepancies. Nevertheless, these findings suggest the profound effects of GSK3 on glucose homeostasis and insulin action through its regulation of pathways in addition to glycogen synthesis.

GSK3 was also reported to negatively regulate the myogenesis process to inhibit skeletal muscle growth by targeting different factors (Figure 1B), such as NFATc3, CUGBP1, and muscle-specific microRNAs.^{61–64} GSK3 inhibition (Lithium at 10 mM for >24 h) was also reported to mimic the activation of Wnt signaling to cooperate with insulin in the induction of myotube fusion and the hypertrophy of muscle satellite cells.^{65,66} Thus, the inhibition of GSK3 may be a potential therapeutic strategy to prevent the development of myotonic dystrophy by increasing myotube formation.⁶⁴ Moreover, GSK3, particularly GSK3 β is required for the induction of muscle atrophy and its deletion or inactivation facilitates muscle mass recovery from atrophied skeletal muscle.^{67–69} Interestingly, GSK3 activity in rat skeletal muscle is significantly reduced by physical exercise,⁷⁰ which may trigger the initial event that results in enhanced muscle protein metabolism and muscle function involved in energy metabolism regulation. GSK3 β absence or inhibition (CHIR99021 at 7 μ M for 48 h) also enhances skeletal muscle mitochondrial oxidative metabolism in skeletal muscle *in vitro* and *in vivo*,⁷¹ which provides another layer of evidence suggesting that the function of GSK3 in skeletal muscle is negatively associated with energy homeostasis.

3 | GSK3 IN THE LIVER

Global GSK3 α -knockout mice showed improved glucose intolerance and insulin resistance, although it was observed only with the ICR outbred strain, not with the C57BL/6 inbred strain, suggesting a strain-specific effect of GSK3.^{12,58} Enhanced hepatic glycogen deposition along with increased PKB phosphorylation and IRS-1 expression in the liver of global GSK3 α knockout mice was correspondingly observed. In hepatic cells *in vitro*, the GSK3 inhibitors (30 μ M of SB216763, SB415286 and 20 mM Lithium for 3 h) were reported to decrease glucose production by reducing the expression of PEPCK and G6Pase genes, encoding two vital factors for gluconeogenesis and regulated by insulin in a glycogen synthesis-independent manner.⁷² The long-term treatment (daily for 3 weeks) of Lep^{ob/ob} mice using a selective peptide inhibitor of GSK3, L803-mts, reduced blood glucose levels and improved glucose tolerance accompanied by increased IRS-2 expression but decreased activity of hepatic gluconeogenic factor CREB and its target gene PEPCK (Figure 1B).⁹ Although GSK3 β may dominantly function in skeletal muscle to regulate glucose metabolism and thus affect whole-body glucose homeostasis,³ notably, the treatment of Zucker diabetic fatty (fa/fa) rat models with GSK inhibitors CHIR98023 and CHIR99021 (single oral dose at 30 mg/kg) led to enhanced glucose disposal with increased glycogen synthesis in the liver but not in the muscle of the mice.² This finding indicates that glycogen synthesis in muscle might not contribute to the acute effects of these two inhibitors on the whole-body glucose metabolism unlike in the liver. Both observed sustained (from chronic GSK3 inhibition and genetically modified models) and acute effects (from transient GSK3 inhibition) of GSK3 in the liver propose that GSK3 especially GSK3 α in the liver plays a critical role in regulating glycogen synthesis and glucose metabolism. However, unexpectedly, neither improved insulin sensitivity nor glucose homeostasis was observed in mice with hepatic-specific deletion of GSK3 α , including those with an ICR background, as observed in mice with muscle-specific deletion of GSK3 α .^{12,58} Similarly, no altered glycogen deposition, glucose metabolism, or insulin sensitivity was observed in mice with liver-specific GSK3 β conditional knockout, in contrast to muscle-specific GSK3 β -depleted animals, which displayed improved glucose tolerance and enhanced insulin action, including increased glycogen deposition.³ These studies suggest that the effects of GSK3 α on glucose metabolism and insulin action are sensitive to genetic background and/or may depend on tissues other than skeletal muscle and liver. It is interesting to consider which tissues may be critical for the whole body effects when GSK3 in the muscle and liver is not the primary contributor to glucose metabolism. Although the details of the mechanisms by which GSK3 orchestrates all

the functions in tissues, including liver, skeletal muscle, and other tissues, to regulate glucose metabolism and insulin action through glycogen synthesis or other functions have not been fully elucidated, studies provide strong evidence indicating that the influence of GSK3 α and GSK3 β on glucose metabolism is complicated and may be tissue-specific and strain- or even species-dependent. Some unknown substrates of GSK3 in addition to its long-recognized substrates, such as GS, may also play critical roles in regulating the metabolic phenotype.

Interestingly, similar to the increased GSK3 α inactive phosphorylation in skeletal muscle with GSK3 β specific deletion,³ the inactive phosphorylation of GSK3 β in the liver of GSK3 α knockout mice was also observed to be enhanced.¹² The augmented insulin signaling resulting from disrupted GSK3 α or GSK3 β action in the liver or muscle might be the cause of the attenuated activity of the undisrupted GSK3 isoform, as indicated by increased inactivating phosphorylation. These observations indicate that one isoform does not compensate when the other isoform is inactivated in its primary functional tissue. The reason for the functional dominance of these two GSK3 isoforms remains unclear. The interaction between GSK3 isoforms and tissue-specific factors may play a critical role in regulating isoform dominant effects in tissues. The noncatalytic domains within GSK3 may be critical for the interaction and subsequent effects on functions not related to its GS kinase activity.

4 | GSK3 IN ADIPOSE TISSUE

The dysfunction of adipocytes and dysregulation of adipocyte differentiation play critical roles in the development of obesity and its associated disorders. In fat tissues isolated from HFD-fed obese mice, GSK3 activity was found to be elevated.⁵⁶ This abnormally elevated GSK3 expression in adipose tissues is thought to be associated with obesity and its associated diseases, such as insulin resistance and T2D. Interestingly, skeletal muscle-specific overexpression of GSK3 β caused an increase in mouse fat mass,⁵⁷ suggesting communication between tissues to collectively contribute to energy homeostasis.

The expression of GSK3 in adipose tissue is more closely linked with changes in body weight than with glycogen synthesis or insulin action.⁵⁵ This outcome is not surprising because some previous studies have revealed that protein phosphatase-1 can be the primary kinase regulating GS activity, not GSK3 in adipose tissue,^{73,74} implying that GSK3 might play other physiological functions in adipose tissue. Some evidence suggests that GSK3 indirectly influences insulin action and glucose metabolism by regulating adipocyte differentiation, fat mass, the local microenvironment, inflammation, etc (Figure 1B). Some published data demonstrate that a GSK3 inhibitor blocked 3T3-L1 preadipocyte adipocyte differentiation.⁷⁵⁻⁷⁷ It was also demonstrated that GSK3 positively contributes to adipogenesis by upregulating the expression and activity of STAT5 and SRF1.⁷⁵

GSK3 also participates in the regulation of adipogenesis via other pathways, such as Wnt/ β -catenin signaling and phosphorylation of C/EBP α and C/EBP β .^{75,77-80} The adipogenic differentiation of human adipose tissue-derived stem cells was also found to be positively regulated by GSK3, although the mechanism was not elucidated.⁸¹ In addition to Ser9 inactivating phosphorylation, phosphorylation at the Thr356 residue of GSK3 β by tyrosine phosphorylation-regulated kinase 1A (Dyrk1A) kinase, which has dual specificity and inactivates GSK3 β , was demonstrated to contribute to the obesity-resistant phenotype, and the dephosphorylation of Thr356 was required for adipogenesis *in vitro*.⁸²

In contrast to white adipocyte differentiation, the brown adipocyte-mediated thermogenic program stimulated by β -adrenergic signaling, which can protect against obesity and its associated metabolic disorders. GSK3 was found to negatively affect basal and β -adrenergic stimulated thermogenesis (Figure 1B),⁸³ which may be another possible mechanism by which the overactivity or excess expression of GSK3 may have harmful effects on glucose metabolism and insulin action.

GSK3 is also associated with increased inflammation in adipose tissue. GSK3 inhibition in human adipose tissue downregulated proinflammatory cytokines and chemokines.⁸⁴ Chronic treatment of obese mice with GSK3

inhibitors significantly reduced obesity-induced WAT inflammation and attenuated glucose intolerance and insulin resistance.⁸⁵

5 | GSK3 IN THE PANCREAS

Pancreatic β -cell dysfunction and failure directly lead to the development of diabetes. Therefore, the regeneration of the β -cell mass and recovery of the function in diabetic patients is a promising approach for treating patients. Mussmann et al.⁸⁶ reported that GSK3 inhibition protects β cells against cell death induced by high concentrations of glucose and saturated fatty acid palmitate and stimulates β -cell replication.^{86,87} Consistently, the depletion of GSK3 β in *Irs2*-knockout mice prevented the onset of diabetes, which is potentially caused by increased β -cell proliferation and the prevention of increased apoptosis (Figure 1B).⁸⁸ Moreover, mice with GSK3 β depleted in β cells displayed specifically increased β -cell proliferation and expanded mass and, importantly, resistance to fat diet-induced diabetes.^{89,90} Although it is not clear whether GSK3 α functions the same as GSK3 β in β cells, we cannot exclude this possibility considering that the improved metabolic phenotype of GSK3 α -knockout mice is not acquired directly from the liver or skeletal muscle. Interestingly, endoplasmic reticulum (ER) stress- and oxidative stress-induced β -cell apoptosis requires the activation of GSK3. GSK3 inhibition increases β -cell survival by increasing the stability of factors such as IPF1/PDX1, which are crucial in pancreas development and β -cell function.^{91,92} The positive effect of GSK3 inhibition on β -cell proliferation was also confirmed in human beta cells. These studies highlight a practical application of GSK3 inhibition in beta-cell regeneration and its potential in diabetes treatment.⁹³

6 | GSK3 IN THE BRAIN

Emerging evidence has suggested that neuronal signaling plays important role in glucose homeostasis and insulin action in peripheral tissues. Indeed, food intake patterns and physical activity regulated by the neuronal system may substantially affect metabolic phenotypes by changing body weight. Moreover, hypothalamic insulin signaling was reported to be very important for whole-body glucose homeostasis and especially hepatic glucose production.^{94–96} In addition, peripheral tissues, such as adipose tissue, secrete many adipokines to communicate with the neuronal system to collectively affect the metabolic phenotype of the whole body.

In adulthood, both GSK3 α and GSK3 β are expressed in the adult mouse brain and are particularly enriched in the hippocampus, neocortex, and cerebellum.⁹⁷ The loss of GSK3 α in mice resulted in decreased body weight, improved insulin resistance, and increased leptin, an adipokine that can be regulated by the neuronal signaling pathway.¹² However, neither improved glucose tolerance nor insulin action was observed in mice with liver- or muscle-specific deletion of GSK3 α ,⁵⁸ making feasible the hypothesis suggesting that the effects of GSK3 α on metabolic phenotype are not derived directly from liver or skeletal muscle but are mediated through other tissues. It remains unknown which tissue type is critical for the observed improvement of glucose tolerance and hepatic insulin sensitivity. One possibility is that GSK3 α loss may indirectly act on neuronal metabolism by affecting insulin signaling and leptin signaling to contribute to the improved acquisition of the whole body and liver-specific metabolic phenotype observed in global GSK3 α -KO mice. It has been established that neuronal insulin signaling plays a critical role in maintaining whole-body energy homeostasis by impacting the function of peripheral tissues, including the liver, skeletal muscle, and adipose tissue. Adipocyte-secreted leptin primarily acts on the hypothalamus to regulate food intake, thermogenesis, and glucose metabolism. The improved insulin action accompanied by decreased body weight and increased leptin in GSK3 α mice provides some support for the suggestion that GSK3 α within the brain impinges on the whole body and liver-specific function. Another possibility is that GSK3 directly functions in the brain regions that are associated with energy metabolism regulation. Corroborating this view, the

brains of STZ-induced diabetic animals show increased expression and activity of GSK3.⁹⁸ In particular, GSK3 β is overactive in the hypothalamus of leptin-deficient obese mice. Intracerebroventricular injection of a GSK3 β inhibitor improved glucose homeostasis. The neuron-specific overexpression of functional GSK3 β in the hypothalamic ARC, a key brain region for neuronal control of energy and glucose homeostasis, significantly impaired glucose tolerance and increased the food intake and body weight in HFD-fed mice (Figure 1B). Central inhibition of GSK3 β in Lep^{ob/ob} mice also led to reduced hepatic gluconeogenesis and downregulated expression of the gluconeogenesis-associated gene, PEPCK,⁹⁹ which provides evidence that the activity of GSK3 in the neuronal system regulates peripheral glucose homeostasis. This study also prompted us to hypothesize that the improved metabolic phenotype in GSK3 α -knockout mice may be associated with its function in the brain since no changes were observed in animals with liver- or skeletal muscle-specific deletions. Interestingly, GSK3 may contribute to obesity-induced inflammation in the hypothalamus (Figure 1B).⁹⁹ In addition, GSK3 contributes to obesity-induced adipose tissue inflammation, it is plausible that GSK3 may mediate the cross-talk between components in the brain and adipose tissue by regulating inflammation-related factors. Despite the potential that other types of tissues may be involved in communication with the brain in addition to those under GSK3 control, it is still tempting to conclude that neuronal or hypothalamic GSK3 activity potentially influences whole-body glucose metabolism and energy homeostasis either through its cooperativity with other signaling pathways, such as insulin and leptin signaling pathways, or possibly by regulating other currently unknown substrates.⁹⁹

GSK3 contributes to physical activity modulation by affecting energy expenditure in brain cells. Martin et al.¹⁰⁰ reported that GSK3 β regulates brain mitochondrial and NAD metabolism. In particular, GSK3 β inhibition promotes mitochondrial energy metabolism in neuronal and glioma cells by increasing PGC1 α protein stability, nuclear localization, and transcriptional activity. This effect of GSK3 β is region-specific and is mainly limited to the hippocampus. GSK3 inhibition was demonstrated to have a neuroprotective effect by reprogramming the metabolism of neuronal cells, as indicated by increased glycolysis. This effect is mediated partly by increased hexokinase II in the mitochondria of neuronal cells.¹⁰¹

Some studies report that GSK3 regulates brain developmental processes such as neuronal progenitor proliferation, neuronal polarity, neurogenesis, axon growth, and neuroplasticity by modulating upstream and downstream signaling pathways (Figure 1B), including the PI3K/AKT and Wnt pathways, β -arrestin2/PP2A pathway, and Notch pathway.^{33,102–107}

7 | GSK3 IN CARDIAC TISSUE

Since the first study that reported GSK3 β acts as a negative regulator of cardiac hypertrophy, numerous studies,^{108,109} using various genetically edited animal models have demonstrated that both GSK3 α/β are important regulators of cardiac biology and pathophysiology. The comprehensive roles of GSK3 signaling in myocardial function and diseases have been reviewed previously and the readers can refer to a review for details.⁴³ In brief, it seems that deletion of GSK3 α/β specifically in cardiomyocytes (CMs) is protective in response to chronic myocardial infarction. However, GSK3 β mediated modulation of cardiac function in ischemic heart is complex and context-dependent. Some contradictory observations were reported that both overexpression/activation and sustained systemic inhibition of GSK3 β could be detrimental. Interestingly, some studies suggest that GSK3 α/β may negatively regulate cardiac hypertrophy under both normal and pathological conditions. GSK3 α works as a negative regulator of hypertrophy via modulating mTORC1 and autophagy at baseline.⁴² But constitutively activated GSK3 α could be detrimental via repressing CM proliferation especially under stresses such as pressure overload.^{110,111} Well-compensated cardiac hypertrophy due to inhibition of GSK3 β at baseline could be beneficial to cardiac function.¹¹² But GSK3 β may not be a dominant regulator of transverse aortic constriction-induced hypertrophy.¹¹³ Nevertheless, GSK3 β is required for cardiac development and CM proliferation (although the role of GSK3 in the

congenital heart remains to be studied),⁴³ GSK3 α could retard the aging process as global deletion of GSK3 α accelerates age-related pathologies in multiple tissues, which is also associated with dysregulation of mTORC1.⁴¹

GSK3 is also considered a negative regulator of fibrosis, which is essential to protect the heart against ischemic injury. The inhibitory effects of GSK3 β on fibrosis are mediated by Wnt/ β -catenin-dependent and TGF β 1-SMAD3-dependent mechanisms.⁴³ Both GSK3 isoforms can participate in the regulation of glucose metabolism by functioning on GS in cardiac tissue, although GSK3 β plays a more prominent role.¹¹⁴ Cardioprotection and improved recovery of postischemic left-ventricular function can be achieved by inhibiting GSK3, which increases glycogen synthesis and reduces glycolysis.¹¹⁵ GSK3 function in chronic obesity-induced cardiovascular diseases was also reported. The CM-specific deletion of GSK3 β led to cardiac dysfunction and heart failure in obese animals fed an HFD without affecting body weight, fat mass, and other metabolic parameters such as cholesterol, insulin, and blood glucose.¹¹⁶ However, deleting CM-GSK3 β after established obesity (a clinically more relevant model) did not adversely affect cardiac function, but improved glucose tolerance.¹¹⁷ Consistently, chronic treatment of GSK3 inhibitor in established HFD induced obese rats improved insulin resistance without exacerbating obesity-induced cardiac hypertrophy.¹¹⁸

GSK3 activity is also associated with diabetes-related changes in cardiac energy metabolism. GSK3 β activity was found to be increased in the heart of diabetic mice and to contribute to the imbalance of glucose/lipid metabolism, i.e., decreased glucose metabolism but enhanced lipid accumulation in the heart (Figure 1B). Consequently, it causes cardiac inflammation and oxidative damage, which eventually leads to cardiac dysfunction. PGC1 α was found to be negatively regulated by overactivated GSK3 in the heart, which could partially lead to the pathogenesis of diabetic cardiomyopathy.¹¹⁹

8 | GSK3 AND TUMOR METABOLISM

Tumor cells usually display increased glucose uptake and compete for glucose much more intensely than normal cells. Apart from stimulating tumor growth, increased glucose uptake and anaerobic metabolism also contribute to the invasion and metastasis of tumor cells. The overexpression and positive effect of GSK3 on tumors have been observed in certain types of cancers, including colon, liver, ovarian, and pancreatic tumors.^{120–123} In addition, glycogen synthesis is significantly decreased in aggressive cancer cells, probably owing to GSK3 activation, which also promotes the utilization of glucose for energy production. Therefore, the inhibition of GSK3 may lead to decreased cancer cell proliferation and survival because it would upregulate glycogen synthesis.¹²⁴

However, the negative effects of GSK3 on tumors have also been substantially reported. The best-known tumor-suppressing effect of GSK3 involves the suppression of the Wnt/ β -catenin pathway, which results in the destabilization of β -catenin, decreases c-Myc, cyclin-B1, and survivin expression, and restricts cancer cell proliferation and tumorigenesis.^{125,126} In another example, GSK3 was reported to be activated by PP2A upon metformin treatment, which activates PP2A by inhibiting CIP2A, the suppressor of PP2A. Activated GSK3 degrades MCL1, a prosurvival protein. The GSK3-dependent reduction in MCL-1 leads to synergistically mediated metformin/hypoglycemia-inhibited tumor growth and cell death.¹²⁷

In some types of cancer, such as renal cancer, prostate cancer, and breast cancer cells, GSK3 was found to promote cancer cell survival. GSK3 inhibition was able to increase intracellular glucose uptake, but the increased intracellular glucose could not be utilized by cancer cells due to increased glycogen storage, leading to an imbalance in energy homeostasis and autophagy of the cancer cell upon serum starvation.^{128–130} The autophagy thus induced might be mediated by suppressing mTOR signaling, which contributes to tumor growth inhibition. However, in another study, GSK3 inhibition activated autophagy to promote the prosurvival of pancreatic cancer cells despite GSK3 inhibition triggering apoptosis.¹³¹ This outcome is not surprising, as autophagy plays a dual role in tumorigenesis by acting as both a tumor suppressor at an early stage and a tumor promotor at a late stage.¹³² Notably, GSK3 was demonstrated to be required for serum- but not glucose deprivation-induced autophagy in colorectal

cancer cells and contributed to cellular energy homeostasis.¹³³ Although the effect of GSK3 on tumor growth was not illustrated in this study, the GSK3 effect on autophagy is different in other cancers, suggesting a context-dependent role for GSK3 in regulating cellular processes. Importantly, these contradictory studies reveal that GSK3 may have some beneficial impacts to a certain extent. Again, it is plausible that a balance between a normal physiological level and a deleterious level of GSK3 may be critical in determining the outcome in the body.

GSK3 has also been found to play a dual role in limiting tumor cell growth while maintaining tumor cell survival. For example, by repressing c-Myc, an activator of glycolysis, GSK3 limits anti-CD40 + interleukin (IL)-4-stimulated B-cell growth and proliferation through glycolysis restriction. Simultaneously, GSK3 is also required to prevent ROS-induced B-cell apoptosis and promote B-cell survival under glucose deprivation conditions. Interestingly, this effect was also found to be realized through GSK3 repression of c-Myc, which decreases mitochondrial content and limits ROS production.¹³⁴

GSK3 has also been applied as a biomarker in cancer therapy. Momcilovic et al.¹³⁵ reported that chronic treatment of lung SCC by the mTOR inhibitor MLN128 suppressed glycolysis in cancer cells, mitigating the cell-killing effect of MLN128. In addition, these cancer cells maintain a high proliferative rate by adapting to glutaminolysis as an alternative nutrient source despite chronic glycolysis suppression. GSK3 acts as a negative regulator of adaptive glutamine metabolism by degrading c-Myc and c-Jun to downregulate GLS (glutaminase), which will prevent cancer cells from escaping the cell death caused by mTOR inhibition. This conserved metabolic signature was observed in a panel of human cancers showing high glycolysis levels, including SCCs of the lung, head, and neck, and osteosarcomas. Therefore, the negative phosphorylation status of GSK3 α/β at Ser21 or Ser9 may be a predictive biomarker of the response to combined metabolic therapies targeting mTOR and GLS.¹³⁵

8.1 | GSK3 associated diseases and the application of inhibitors

GSK3 has been implicated in many diseases such as metabolic disorders including obesity and diabetes, cardiovascular disease, cancer, neurological disorders including Parkinson's disease (PD), Alzheimer's disease (AD), mood disorders, BPD, etc.^{27,43,53,136,137} Thus, many GSK3 inhibitors have been developed with the potential to treat these diseases and many of them have already been or will be tested in clinical trials (Table 1)^{27,138-142} and database from ClinicalTrials.gov.

According to working mechanisms, GSK3 inhibitors have been classified as (1) ATP-competitive inhibitors, (2) non-ATP competitive inhibitors (including substrate competitive inhibitors), (3) cations, and (4) allosteric inhibitors.^{141,143} As the most widely used GSK3 inhibitor, lithium is the first identified pharmacological GSK3 cation inhibitor. It has been effectively used in the treatment of BOD for decades with encouraging findings as a mood stabilizer. Lithium is suggested to have several molecular targets in BPD, one leading mechanism of action is the inhibition of GSK3 via inhibiting both GSK3 α/β via directly competing for magnesium or indirectly modulating GSK3 phosphorylation in many cell types.¹⁴⁴ Lithium is currently the most commonly used GSK3 inhibitor in clinical trials (Table 1).

Recently, developing GSK3 isoform-selective or substrate competitive inhibitors for the treatment of different types of disease becomes a common interest because of the recognition that complete or dual blocking of both GSK3 isoforms may raise some adverse effects.

The application of isoform-selective inhibitors in some diseases provides strong evidence that it is very promising to develop and optimize GSK3 inhibitors to treat associated diseases. Licht-Murava et al. and others have been trying to develop and test different types of GSK3 inhibitors including substrate competitive inhibitors (may potentially target specific cells and pathways), highly selective isoform inhibitors, and Akt-activated GSK3 β inhibitor.^{136,145,146} The most advanced GSK3 inhibitor, Tideglusib is an irreversible inhibitor of GSK3 β and has been used in clinical trials for several diseases with proven relative safety¹⁴⁷ (Table 1).

TABLE 1 GSK3 inhibitors in clinical trials (as of July 2021)

Name	Conditions	Clinical trials	Study phase	Status
	Treatment-resistant depressive disorder	NCT03004521	Phase 4	Recruiting
	Bipolar disorder	NCT00667745	Phase 4	Completed
	Osteosarcoma	NCT01669369	Phase 4	Recruiting
	Mild cognitive impairment	NCT03185208	Phase 4	Recruiting
	Bipolar disord	NCT00870311	Phase 4	Completed
	Neurocognitive disturbance in HIV infection	NCT01348282	Phase 4	Completed
	Stroke	NCT01112813	Phase 3	Completed
Lithium	Aggressive conduct disorder	NCT00000385	Phase 3	Completed
	Frontotemporal dementia	NCT02862210	Phase 2	Recruiting
	Neuroendocrine tumors	NCT00501540	Phase 2	Completed
	Alzheimer disease	NCT01055392	Phase 2	Unknown ^a
	Brain cancer	NCT01105702	Phase 2	Terminated
	Small cell lung carcinoma	NCT01553916	Phase 1	Completed
	Huntington's disease	NCT00095355	Phase 2	Completed
	Parkinson disease	NCT04273932	Phase 1	Recruiting
	Prostate cancer	NCT02198859	Phase 1	Completed
	Leukemia	NCT01820624	Phase 1	Completed
	Congenital myotonic dystrophy	NCT03692312	Phase 3	Recruiting
	Alzheimer's disease	NCT01350362	Phase 2	Completed
Tideglusib	Autism spectrum disorders	NCT02586935	Phase 2	Completed
(NP12,	Myotonic dystrophy 1	NCT02858908	Phase 2	Completed
NP031112)	Congenital myotonic dystrophy	NCT03692312	Phase 2	Recruiting
	Alzheimer's disease	NCT00948259	Phase 2	Completed
	Pancreatic cancer	NCT01632306	Phase 2	Terminated
LY2090314	Acute leukemia	NCT01214603	Phase 2	Completed
	Advanced or metastatic cancer	NCT01287520	Phase 1	Completed
	Advanced, metastatic salivary gland carcinoma	NCT04832438	Phase 2	Not yet recruiting
	Myelofibrosis	NCT04218071	Phase 2	Recruiting
9-ING-41	Advanced sarcomas	NCT04906876	Phase 2	Withdrawn
	25 Types of cancer	NCT03678883	Phase 2	Recruiting
LY317615	Solid tumor lymphoma, malignant	NCT01388335	Phase 1	Completed
(Enzastaurin)	Advanced cancer, ovarian cancer	NCT00550927	Phase 1	Completed
Tungstate	Obesity	NCT00555074	Phase 2	Completed

Abbreviation: GSK3, glycogen synthase kinase-3.

^aStudy has passed its completion date and status has not been verified in more than 2 years (from [ClinicalTrials.gov](https://clinicaltrials.gov)).

Recently, McCamphill et al. have also developed a set of GSK3 isoform-selective inhibitors and one of the GSK3 α specific inhibitors BRD0705 sufficiently corrected several phenotypes in a fragile X syndrome mouse model without the development of side effects caused by other clinical trial drugs.¹⁴⁸ BRD0705 also displayed great potential in treating acute myeloid leukemia (AML).¹⁴⁹ Unwanted side effects and increased β -catenin were not observed in both cases alongside, indicating the valuable therapeutic potential of GSK3 isoform-selective inhibitors in neurological diseases and cancer treatment. L803-mts, a substrate competitive GSK3 inhibitor, was found to meliorate cognitive deficits and showed beneficial effects for AD and is undergoing preclinical test.¹⁵⁰ Some other GSK3 inhibitors are also under preclinical studies such as SB415286, 6-BIO, AR-A014418, and some natural products^{137,150}

9 | GSK3 INHIBITOR AND NEURODEGENERATIVE DISEASES

More than 100 proteins have been proposed to be substrates for GSK3 in rodents and humans such as Tau protein and A β (amyloid β peptide), two key factors involved in the pathogenesis of AD. The abnormal overactivation of GSK3 is usually observed in the brains of neurodegenerative patients, which leads to hyperphosphorylation of key proteins implicated in neurodegenerative diseases such as Tau protein. GSK3 modulates Tau function via phosphorylating it, disrupting its transcription and stimulating its aggregation, leading to disrupted brain cell function.¹⁴⁴ Furthermore, A β activates GSK3 to contribute to the effects of GSK3 on Tau protein.¹⁵¹ A potential role of Tau in PD was also reported, as is GSK3, whose transcription and splicing were changed by PD. GSK3 promoted apoptosis results in neuronal loss and death and contributes to the development of PD and AD while GSK3 inhibitors could counteract the neuronal loss in PD.^{40,53,136} Some beneficial effects of lithium on Tau and A β pathology were also observed. These studies propose GSK3 inhibition as a therapeutic strategy for AD and PD and other associated neurodegenerative diseases.

While some conflicting results were reported on the therapeutic effect of lithium on AD models, some positive results including several clinical trials (although not in all studies), warrant the continuous exploration of the therapeutic potential of lithium in neurodegenerative diseases. For example, Phase 2 and Phase 1 studies of lithium for AD and PD are undergoing (Table 1). Phase 2 clinical studies of Tideglusib (NP-12, NPO31112), one of the non-ATP-competitive GSK3 inhibitors, were also conducted for AD treatment without strong adverse effects to be observed (Table 1).^{147,152}

Growing evidence demonstrated that GSK3 inhibition may also be effective for other neurological and psychiatric disorders. It is not surprising to observe GSK3 inhibitors ameliorate these neurological and psychiatric disorders considering the anti-inflammatory, antiapoptosis, and neuroprotective effects of GSK3 inhibitors as most of these diseases are associated with inflammation, apoptosis, and neurodegeneration. Despite this, more studies are necessary and warrant the efficiency and safety of GSK3 inhibitors.

10 | GSK3 INHIBITORS AND CANCER

A recent study that GSK3 α selective inhibitor BRD0705 exhibited a beneficial effect in treating AML encourages researchers to identify more optimized strategies to target GSK3 for cancer treatment.¹⁴⁹ Looking back, the application of GSK3 inhibitors in cancer is complicated and cancer type-dependent. The involvement of GSK3 in cancer development is mainly through its interaction with the variable pathways such as PI3K/Akt, Wnt/ β -catenin, nuclear factor kappa B (NF- κ B), MAPK, ERK1/2 pathways.¹⁵³ Owing to the complexity of the interactions between GSK3 and the other different pathways, whether GSK3 is a tumor suppressor or oncogene is context-dependent. For example, by phosphorylating β -catenin, GSK3 could behave as a tumor suppressor by directing β -catenin degradation and repressing tumorigenic effect (mainly via shutting down its downstream cancer genes like cyclin

D1, Myc, and c-jun.¹⁵⁴ However, GSK3 also shows increased expression in some types of cancer and associates with tumor cell proliferation.¹⁴¹ The controversial effects of GSK3 on apoptosis are another underlying mechanism leading to the dual role of GSK3 in tumorigenesis.¹³⁶

Despite the controversial role of GSK3 in cancer development, many GSK3 inhibitors have been conducted for preclinical studies using *in vitro* and *in vivo* animal models with the demonstration of beneficial anticancer effects in some types of cancer such as pancreatic cancer, glioblastoma, leukemia, bladder cancer, etc. Some inhibitors already entered clinical trials. For example, Phase 1 clinical trials have been conducted to evaluate the safety and efficiency of lithium on prostate cancers and leukemia¹⁵⁵ (Table 1). Another two GSK3 inhibitors, LY2090314 and 9-ING-41, have also been tested for treating some types of cancer as a single agent or in combination with other drugs. LY2090314 was safe and well-tolerated and showed, to some extent, an anticancer activity when combined with other agents.^{137,156} Emerging evidence also suggests GSK3 is associated with immune response in cancer and thus shows the potential of being targeted in cancer immunotherapy. GSK3 negatively regulates the response of NK cells and T cells and administration of GSK3 inhibitors enhanced immune cells' cancer cell cytotoxicity and improved their anticancer ability. Three ongoing clinical trials are evaluating the safety and efficiency of NK cells precultured with GSK3 inhibitors CHIR99021. These pretreated NK cells were infused into patients of several types of cancer.¹³⁷ There are also studies suggesting that GSK3 inhibitors may potentiate the therapeutic effect of the immune checkpoint antibodies owing to their regulation of the immune checkpoint gene expression.¹³⁷

11 | GSK3 INHIBITOR AND METABOLIC DISORDERS

We have discussed the pivotal roles of GSK3 in the development of obesity, insulin resistance, T2D, and cardiovascular diseases in previous sections of this review. The involvement of GSK3 in multiple tissues and numerous signaling pathways associated with metabolism and glucose homeostasis warrant the therapeutic potential to targeting GSK3. More and more emerging *in vitro* and *in vivo* preclinical studies also provided substantial evidence that GSK3 inhibitors have beneficial effects in preventing obesity, improving insulin resistance, and T2D as we discussed in the above sections. However, few GSK3 inhibitors were used in clinical trials as antiobesity or anti-diabetic agents. One example is tungstate, an indirect inhibitor of GSK3 via activating (ERK1/2) was used to conduct a Phase 1 clinical trial as an antiobesity agent with demonstrated low toxicity profile.¹⁴² In patients with psychiatric disorders, the administration of lithium lowered blood glucose levels and improved glucose tolerance.^{157,158} Consistently, in patients with types 1 and 2 diabetes, lithium showed a hypoglycemic effect.¹⁵⁸ However, some controversial case reports indicate lithium administration is associated with hyperglycemia.¹⁵⁸ Although whether GSK3 is involved in the lithium-associated hyperglycemia is not clear since lithium may work via a GSK3-independent mechanism, caution still needs to be exercised when using lithium or other GSK3 inhibitors that inhibit total GSK3 for obesity or diabetes treatment. Again, it is critical to develop and test high selective GSK3 isoform-specific or other substrate competitive inhibitors for metabolic disorder treatment, which will help better understand the cellular roles of GSK3 and avoid those off-target effects of these potential pharmacological GSK3 inhibitors.

11.1 | The role of GSK3 in mitochondria

As the powerhouses of mammalian cells, mitochondria are the energy factories that promote the catabolism of substrate molecules to release energy. Therefore, the abundance and the functional activities of the mitochondria are regulated in correlation with the metabolic status of the cells. Mitochondrial dysfunction is associated with many diseases, including cancer, neurodegenerative diseases, cardiovascular disease, stroke, aging, obesity, diabetes, and inflammation. GSK3 regulates the function of mitochondria mainly through two mechanisms. Since most

of the mitochondrial genes are coded in the genome, GSK3 influences mitochondrial activity by indirectly regulating the signaling pathways that guide the expression of mitochondrial genes in the nucleus. Simultaneously, GSK3 was found to be located not only in the cytosol and nucleus but also in mitochondria, indicating that GSK3 may directly influence mitochondrial function.

12 | GSK3 AND PGC1 α , A KEY REGULATOR OF MITOCHONDRIAL BIOGENESIS

PGC1 α is a key regulator of mitochondrial biogenesis and oxidative metabolism to positively contribute to energy homeostasis.^{159,160} The abundant expression of PGC1 α has been observed in tissues with high energy demands, such as skeletal muscle, adipose tissue, heart, brain, and kidney. PGC1 α interacts with many other factors to regulate the expression of genes associated with various energy metabolism programs, such as glycolysis, fatty acid oxidation, and gluconeogenesis, and other processes, including thermogenesis, adipocyte differentiation, and skeletal muscle development. Any dysregulation or dysfunction of PGC1 α and its cofactors might strongly influence whole-body energy homeostasis to trigger the development of metabolic disorders.^{161–167}

GSK3 modulates mitochondrial biogenesis mainly by negatively regulating PGC1 α (Figure 2). Olson et al.¹⁶⁸ reported that the inactivation of GSK3 can stabilize PGC1 α under conditions of oxidative stress and contribute to its accumulation in neurons to ultimately affect brain energy metabolism. Similarly, Martin et al.¹⁰⁰ demonstrated that GSK3 β inhibition increases mitochondrial energy metabolism and alters NAD(P)H metabolism in glia and neurons in culture, and increases brain energy metabolism in the hippocampus region in mice by increasing PGC1 α stabilization and transcriptional activity. Further studies revealed that PGC1 α might be a direct phosphorylation target of GSK3, which leads to increased PGC1 α instability. Moreover, acute stress activates GSK3, which results in the phosphorylation and degradation of PGC1 α in fibroblasts, skeletal muscle, and adipose tissue,¹⁶⁹ suggesting that GSK3 inhibition of PGC1 α is an alternative cellular response to stress. However, with chronic stress such as calorie restriction, GSK3 activity is inhibited and another energy sensor Sirt1 is activated, which stabilizes PGC1 α and results in a sustained increase in the expression of genes involved in energy metabolism, leading to beneficial effects on energy homeostasis.¹⁶⁹

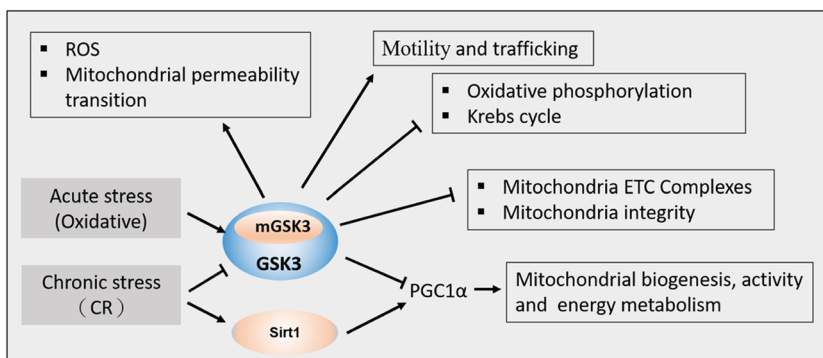


FIGURE 2 GSK3 regulates mitochondrial activity. GSK3 responds to both acute and chronic stresses oppositely. Acute oxidative stress activates GSK3 and results in the inhibition of PGC1 α and mitochondrial activities. Chronic stress such as calorie restriction (CR) inhibits GSK3 but activates Sirt1 to activate PGC1 α that promotes mitochondrial biogenesis and increases energy metabolism. GSK3 including mGSK3 negatively regulates mitochondrial ETC complexes activity, oxidative phosphorylation and mitochondrial integrity but stimulates ROS production and mitochondrial motility and trafficking. ETC, electron transport chain; GSK3, glycogen synthase kinase-3; mGSK3, mitochondrial GSK3 [Color figure can be viewed at wileyonlinelibrary.com]

The GSK3-induced negative regulation of the transcription of PGC1 α , as well as the other PGC1 α signaling networks, including PPAR α / δ , ERR α , Nrf-2 α , and Tfam, was also observed in multiple types of cells, such as endothelial cells, megakaryocytes, vascular smooth muscle and skeletal muscle cells.^{71,170} In addition, GSK3 was shown to negatively regulate the nuclear localization of PGC1 α and the abundance of PGC1 α by promoting its degradation in cells in the absence of Omi, a mitochondrial protease that cleaves GSK3.^{100,171,172}

13 | GSK3 REGULATES MITOCHONDRIAL MOTILITY

GSK3 is also involved in the regulation of mitochondrial motility (Figure 2). For example, it was reported that GSK3 promotes mitochondrial motility in neurons in a Tau protein-dependent manner.¹⁷³ Consistently, CDK5 inhibition causes the activation of GSK3, which leads to stimulated mitochondrial trafficking.¹⁷⁴ GSK3 also acts as a component of the mitochondrial trafficking machinery and associates with TRAK1/NDE1 to promote mitochondrial movement in the mouse brain.¹⁷⁵ However, some conflicting effects of GSK3 on mitochondrial motility have also been reported. Chen et al. reported that GSK3 inhibition increased mitochondrial motility in cultured hippocampal neurons.¹⁷⁶ The mechanism includes the deacetylation of tubulin by HDAC6 whose activity is activated by GSK3 phosphorylation, leading to decreased binding of kinesin-1 to microtubules and reduced mitochondrial transport.¹⁷⁷ These conflicting results indicate that GSK3-regulated mitochondria in the brain might be region-specific.

14 | GSK3 AND MITOCHONDRIAL FUNCTION

The localization of GSK3 especially GSK3 β in the mitochondria has been identified in multiple cell types using different approaches. For example, in human neuroblastoma SH-SY5Y cells, in rat cortical, hippocampal, and cerebellum neurons, GSK3 β but not GSK3 α was detected using immunoblotting and immunoelectron micrograph.^{34,35,178,178,179} Using western blot and co-immunoprecipitations, GSK3 β was also found to translocate into mitochondria mediated by translocase of the mitochondrial outer membrane 20 (TOM20) upon ischemia/reperfusion in CMs.¹⁸⁰ The mitochondrial activity of GSK3 in CMs could be regulated by protection signaling. In CMs, Akt-mediated Ser9 inhibitory phosphorylation of GSK3 β in mitochondria is associated with CM protection against oxidant-induced apoptosis.^{36,181} In human hepatoma cells, the localization of GSK3 β in mitochondria was also observed.¹⁸² These studies imply that mitochondrial GSK3 (mGSK3) may contribute to the regulation of mitochondrial activities. For example, mGSK3 may regulate energy homeostasis by influencing the activity of complexes in the electron transport chain (ETC). It was reported that mGSK3 β inhibited NADH: ubiquinone oxidoreductase, i.e., respiratory chain complex I, leading to reduced oxidative phosphorylation and enhanced ROS production.^{183,184} Moreover, mGSK3 β inactivates pyruvate dehydrogenase to modulate the Krebs cycle.¹⁷⁹ It was also reported that mGSK3 may also interact with other complex subunits such as ETC complexes and mitochondrial permeability transition (MPT) pore complex to regulate mitochondrial activity and function such as mitochondrial oxidative phosphorylation respiration, MPT, mitochondrial integrity, etc.^{172,180,184,185} (Figure 2). Despite these studies, however, we cannot exclude the possibility that some of these effects could result from the regulation of GSK3 on PGC1 α and other downstream substrates or interacting partners of GSK3 in cytosol and nucleus.

14.1 | GSK3 and metabolism related stress responses

As a kinase, GSK3 plays important role in response to different stresses in many types of cells.

15 | GSK3 AND HYPOXIA

Mammalian cells have developed response mechanisms to a low-oxygen environment termed hypoxia, and they are crucial for cell survival when the O₂ supply is inadequate since hypoxic stress can trigger apoptosis. The key factor that orchestrates cell oxygen homeostasis and enables it to adapt to low oxygen levels is the hypoxia-inducible factor (HIF). Acting as a transcription factor, HIF, especially HIF-1, the best-characterized member of the HIF family members, was reported to regulate the expression of more than 100 genes through binding to hypoxia-response elements of target genes to influence many cellular processes, such as angiogenesis, cell proliferation and survival, tumorigenesis, and energy metabolism.

Some reports demonstrated that the inhibition or depletion of GSK3 results in increased HIF-1 α accumulation and hence its target factors including VEGF and GLUT1 proteins, whereas the overexpression of GSK3 leads to the opposite effect.^{186–190} GSK3 negatively regulates HIF-1 α levels by directly phosphorylating HIF-1 α , which is followed by Fbw7-mediated ubiquitination and proteasomal degradation of HIF-1 α in a process that is independent of VHL-mediated hydroxylation.^{187–190} The identification of the residues in HIF-1 α that are phosphorylated by GSK3 further confirmed that GSK3 promotes HIF-1 α degradation.^{187,190} GSK3 repression of HIF-1 α has an important influence on cancer development. HIF-1 α dependent angiogenesis and cell migration were found to be decreased by GSK3 mediated HIF-1 α degradation.¹⁸⁹ In another independent cancer study, GSK3 also repressed HIF-1 α by regulating its translation in human colon carcinoma cells.¹⁹¹ These studies suggest that GSK3 might regulate HIF-1 α activity through more than one mechanism (Figure 3).

In other studies, however, hypoxia-activated GSK3 was found to mediate hypoxia (0.01% O₂-6h)-induced cell apoptosis in vascular smooth muscle cells. GSK3 inhibition prevented this apoptosis.¹⁹² The activation of GSK3 by hypoxia (1% O₂-16h) was also observed in other cell types, such as HepG2 cells,¹⁸⁸ and in the mouse brain and the rat hippocampal CA1 region in vivo (acute exposure to 10% O₂), as indicated by the observed dephosphorylation of

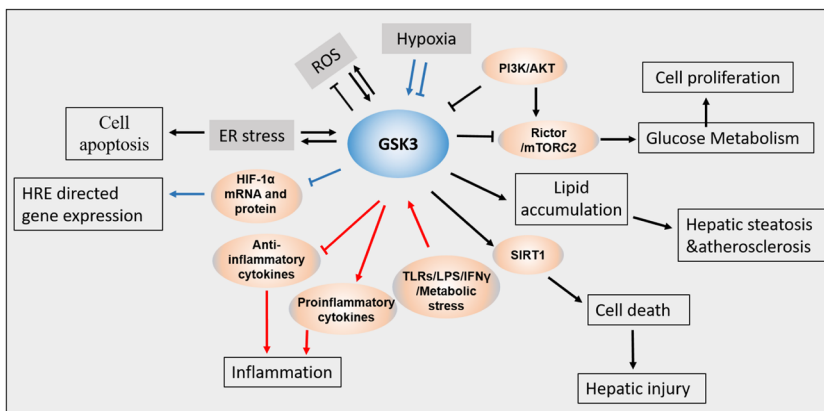


FIGURE 3 The interaction of GSK3 and stresses. A schematic illustration of GSK3 and stresses. GSK3 mediates several downstream effects of ER stress including the inhibition of cell proliferation through inhibition of PI3K/AKT and mTORC pathways, the promotion of lipid accumulation in hepatic cells that results in the hepatic steatosis and atherosclerosis; and activation of Sirt1 which results in the cell death and hepatic injury. ER stress-activated GSK3 also stimulates inflammation. In turn, GSK3 can also promote ER stress to induce apoptosis. Responding to metabolic stress and inflammation mediators including TLRs, LPS, and IFN γ , GSK3 is activated to contribute to inflammation via promoting the expression of proinflammatory cytokines and repressing anti-inflammatory cytokines (red line). While some discrepancy remains, potential feedbacks between hypoxia (blue line) or oxidative stress (ROS) and GSK3 are reported. Correspondingly, HIF-1 α stability is modulated by GSK3 at both mRNA and protein levels. ER, endoplasmic reticulum; GSK3, glycogen synthase kinase-3; HIF, hypoxia-inducible factor; mRNA, messenger RNA; TLR, Toll-like receptor [Color figure can be viewed at wileyonlinelibrary.com]

GSK3 β at Ser9 in HepG2 cells (Figure 3).^{193,194} In contrast, Beiner-Johnson et al. reported that hypoxia (1% O₂-6h) might inactivate GSK3 because the inhibitory phosphorylation of GSK3 was observed in PC-12 cells under hypoxic conditions.¹⁹⁵ Chen et al.¹⁹⁶ also reported that severe hypoxia (0.01% O₂-1h) induced the phosphorylation of GSK3 α and GSK3 β at the inhibitory phosphorylation residues Ser21 and Ser9, respectively, in human HT1080 cells. It is still unclear whether the discrepancy is related to hypoxia severity, and/or exposure duration, or by cell type.

16 | GSK3 AND ROS

An established view suggests that energy metabolism is closely linked to ROS production, and some important enzymes involved in metabolic pathways affect or can be affected by cellular redox status. ROS production is associated with the action of nutrients (such as glucose and lipids) and hormones (such as leptin and insulin), and ROS release greatly influences metabolic regulation by affecting energy metabolism and food intake to ultimately participate in the development processes of metabolism-associated diseases such as obesity, insulin resistance, and diabetes. Mitochondria are the main producers and targets of ROS, which play critical roles in oxidative homeostasis.¹⁹⁷

GSK3 is involved in homeostatic redox equilibrium. Through altering/promoting mitochondrial ROS levels, GSK3 activity is involved in the resultant increase in MPT (Figure 2). MPT refers to increased permeability to the solutes of the inner mitochondrial, and ROS are known to induce MPT and thus cause cell death.^{181,184} GSK3 stimulates ROS production by targeting complex I or possibly by interacting with complex III subunit Rieske, which stimulates ROS signaling in the mitochondrial intermembrane space and cytosol.^{198,199} Thus, GSK3 inhibition can protect cells from MPT- and ROS-induced apoptosis and increase cell survival. Notably, GSK3 activity was increased by ROS in multiple cell types, and this increase is important for ROS-induced cell death in damaged/injured tissue, including heart, neuron, and liver.²⁰⁰⁻²⁰⁴ These studies provide some evidence for potential positive feedback between ROS and GSK3 that controls cell death (Figure 3).

However, some papers reported that GSK3 inhibition may stimulate ROS production in a different set of cell types. For example, GSK3 inhibition increases ROS in chondrocytes, and the subsequent effects include MPT, mitochondrial and DNA damage, and cell senescence.^{205,206} GSK3 inhibition also increases ROS in Mv1 Lu mink lung epithelial cells by binding to and phosphorylating complex IV subunit 6b. The resultant mitochondrial ROS generation also induces senescence.²⁰⁷ Similarly, pharmacological GSK3 inhibition or GSK3 depletion was observed to increase ROS production and senescence in Chang human cervix carcinoma cells and human diploid fibroblasts.^{208,209} Importantly, the absence of GSK3 can be a determinant factor in cells showing the predominant features of senescence, owing to abnormal anabolism,^{208,209} suggesting that GSK3 modulates metabolic alteration to affect cell death. Despite the disparate observations of different types of cells, GSK3 may function as a redox sensor to regulate cellular pathological and physiological processes, and targeting GSK3 may protect against oxidative stress-induced diseases.

17 | GSK3 AND ER STRESS

ER stress is implicated in various diseases, such as obesity, diabetes, neurodegenerative diseases, cancer, and cardiovascular diseases.²¹⁰ ER stress can trigger some adaptive signaling pathways to contribute to the attenuation of cell stress. These pathways are involved in many cellular processes including energy metabolism and homeostasis.²¹¹⁻²¹³

GSK3 is a potential factor implicated in ER stress pathways that regulates energy homeostasis (Figure 3). ER stress-activated GSK3 β interferes with the mTORC2 signaling pathway by specifically phosphorylating the mTOR2 component rictor, repressing glucose metabolism and inhibiting cell proliferation and tumor growth.²¹²

ER stress-activated GSK3 β also induced lipid accumulation to accelerate the development of hepatic steatosis and atherosclerosis.²¹³ In another study, GSK3 was activated in hepatic cells by ER stress that induced PI3K/AKT pathway inhibition, and Sirt1 was found to be a downstream target of GSK3. An increase in Sirt1 by ER-induced GSK3 resulted in cell death and hepatic injury.²¹⁴ Interestingly, GSK3 was shown to act as a promoter of ER stress to induce apoptosis of human neuroblastoma cells in both a caspase-3-dependent and GADD153-dependent ways.^{92,215} The inhibition of GSK3 switches the fate of the cells toward recovery from ER stress. In the liver, the inhibition of GSK3 reduced ER stress-associated apoptosis, which ameliorated D-GalN/LPS-induced liver injury.²¹⁶ Additional studies reported that GSK3 can mediate ER stress to regulate cell differentiation, inflammation, ischemia reperfusion, etc., suggesting that GSK3 may act as a general mediator of ER stress to regulate various biological and pathophysiological processes.^{217–219} In addition, GSK3 is an important mediator of the effects from different types of stress, such as growth factor deprivation and ER stress, promoting autophagy via the TIP60-ULK1 pathway.^{133,220} Oxidative stress also promotes the activation of GSK3 by inducing ER stress, which eventually leads to autophagy through the TIP60-ULK1 pathway.²²⁰

18 | GSK3 AND ITS FUNCTION IN INFLAMMATION

Inflammation is associated with many prevalent diseases, including psychological disorders, neurodegenerative diseases, obesity, diabetes, and cancer. It is now evident that GSK3 has a close association with inflammation and hence a tight link between GSK3 and inflammatory diseases, which makes GSK3 a promising therapeutic target of these conditions. GSK3 regulates multiple immune cells including macrophages, T cells, dendritic cells, and glial cells by affecting their proliferation, differentiation, survival, migration, and macrophage polarization to be implicated in the disparate inflammatory response.^{26,53,85} Using animal models, several reports suggested the anti-inflammatory actions and beneficial effects of GSK3 inhibitors in multiple inflammatory diseases, such as endotoxic shock, peritonitis, arthritis, colitis, diabetes, and atherosclerosis.^{26,53}

GSK3 mediates several subtypes of Toll-like receptor (TLR)-induced inflammatory responses in human monocytes.^{26,221} GSK3 inhibition reduces the production of many proinflammatory cytokines, such as tumor necrosis factor- α , IL1 β , IL6, and IL12, while enhancing the anti-inflammatory cytokine IL10 (Figure 3). Mechanistically, a GSK3 inhibitor increased IL10 by augmenting the binding of CREB/AP1, which promotes IL10 expression, to the nuclear coactivator CBP while suppressing proinflammatory cytokine expression by blocking the binding of NF- κ B p65 to CBP.^{26,222} GSK3 was also found to promote the JAK/STAT pathway by activating STAT1, STAT3, and STAT5, to modulate the synergistic effect of LPS and IFN γ , and to induce the production of proinflammatory cytokines, such as IL6.^{223,224} Therefore, GSK3 inhibitors are ideal candidate chemicals for shifting the balance from a proinflammatory to an anti-inflammatory response, which may be critical in the treatment of related diseases (Figure 3).

In addition to its role in the TLR signaling pathway, GSK3 is also involved in the Nod-like receptor protein 3 (NLRP3) inflammasome pathway, which drives proinflammatory cytokines, particularly IL-1 β , maturation and secretion.^{225–227} The NLRP3 inflammasome serves as a metabolic stress sensor. For instance, elevated extracellular glucose promotes the formation of the NLRP3 inflammasome, which is closely associated with the occurrence of metabolic syndrome. In addition, NLRP3-promoted IL-1 β is considered a risk factor for the development of T2D and contributes to insulin resistance.²²⁸ Thus, NLRP inflammasome activation and elevated IL-1 β levels are implicated as important drivers of the progression from obesity to T2D.²²⁹

Both the NLRP inflammasome and TLR signaling pathways can be activated by stresses such as pathogen infection, psychological stress, ROS stress, and metabolic stress (such as high levels of free fatty acids, glucose, and cholesterol). Increased expression of TLRs (especially TLR2 and TLR4), NLRP3, and the respective ligands in multiple tissues, including adipose tissue, skeletal muscle, and hypothalamus, has been observed in obese/diabetic patients,²³⁰ which suggests the contribution of TLRs and NLRP3 to inflammation initiation and progression. GSK3 is

also activated in response to metabolic stresses. Thus, stress-activated GSK3 may serve as a mediator of both NLRP3 and TLR inflammatory signaling to increase proinflammatory cytokine production and inflammatory response.^{225–227,230}

18.1 | GSK3 and the metabolic regulators

18.1.1 | GSK3 and leptin

Leptin is a peptide hormone secreted by adipocytes. Leptin enters the circulation and binds to its receptor, which is mainly expressed in brain neurons such as the hypothalamus, to regulate food intake and energy homeostasis. The deficiency of leptin and/or its receptor leads to severe obesity and associated abnormalities.²³¹ The cross-talk between leptin and GSK3 has been observed in different types of tissues under different physiological and pathophysiological conditions, which indicates complex cross talk (Figure 4).

In many insulin-responsive tissues, leptin sensitizes the tissue response to insulin and promotes glucose homeostasis. Several studies have shown that in pancreatic β cells, leptin activates AMPK to indirectly activate GSK3 β . Then, GSK3 β phosphorylates and inactivates PTEN, which promotes ATP-sensitive potassium (K_{ATP}) channel trafficking to stimulate insulin secretion.^{232–234} In hypothalamus cells, leptin also depends on maintained GSK3 to phosphorylate and inactivate PTEN to open K_{ATP} channels in vitro,²³⁴ which is opposite to the sustained inhibition of GSK3 by insulin. Further characterization of the GSK3 phosphorylation status revealed that leptin induces only transient inactivation of the serine phosphorylation of GSK3 but sustainably increases GSK3 active tyrosine phosphorylation (Tyr-216/Tyr-279) in hypothalamus cells, with the outcome being maintained GSK3 activity.²³⁴ In human Mesenchymal Stem Cells (hMSCs), activation of GSK3 by leptin mediates the effect of leptin in maintaining mitochondrial integrity and enhancing mitochondrial function in hMSCs, contributing to the efficacy of hMSC-based therapy of cardiovascular diseases by increasing hMSC survival.²³⁵ In contrast, in the mouse cerebral

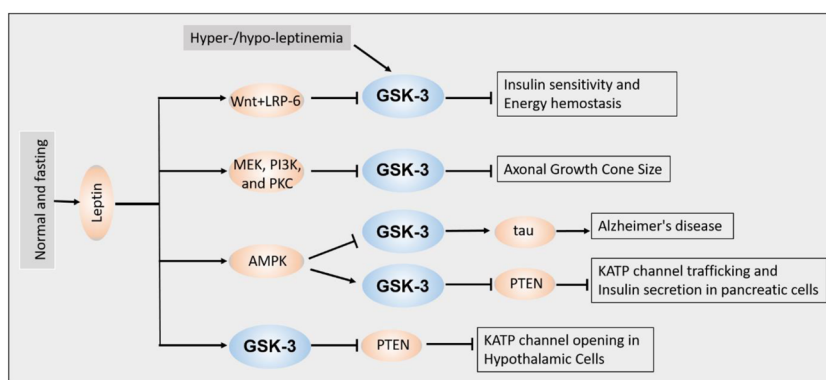


FIGURE 4 The crosstalk between GSK3 and leptin signaling. In hypothalamus cells, leptin depends on activated GSK3 by phosphorylation at Tyr-216/Tyr-279 to phosphorylate and inactivate PTEN to open K_{ATP} channels. In pancreatic β cells, leptin also activates GSK3 via AMPK to inhibit PTEN. KATP is the downstream target of PTEN and is activated by leptin that finally activates insulin secretion. However, Normal physiological levels of leptin in leptin-sensitive animals activate Wnt/LRP-6, leading to the inactivation of GSK3 in the hypothalamus, which sensitizes insulin signaling and contributes to energy homeostasis. Leptin also inactivates GSK3 to inhibit the phosphorylation of Tau protein. In contrast, upon leptin deficiency or hyperleptinemia in leptin-resistant obese animals, leptin activates GSK3 in autocrine and indirectly neuroendocrine manners, which exacerbates obesity-induced insulin resistance and glucose intolerance. GSK3, glycogen synthase kinase-3 [Color figure can be viewed at wileyonlinelibrary.com]

cortex, leptin induces growth cone expansion by inactivating GSK3 β ,²³⁶ which suggests that leptin may have a dual effect on GSK3 activity under different conditions. Hyperphosphorylated Tau is closely associated with neurodegenerative diseases, and GSK3 is a recognized kinase that phosphorylates the Tau protein in neuronal cells. In SH-SY5Y cells, a model for neurodegenerative disorders, leptin was also found to inactivate GSK3, which reduced the level of hyperphosphorylated Tau protein.²³⁷ These results suggest that leptin might lead to different GSK3 statuses and generate distinct physiological outcomes in a context- or tissue-dependent manner. In fact, in aged and/or obese animals, abnormally elevated leptin was reported to impair the insulin-triggered inactive phosphorylation of GSK3 and inhibit the early step of insulin signal cascade such as MAPK activation, leading to insulin-desensitizing effects in white adipocytes, suggesting that long-term hyperleptinemia is associated with high GSK3 activity under aging or obesity conditions.²³⁸ Increased GSK3 activity in the hypothalamus of lep^{ob/ob} mice exacerbated the development of obesity and glucose intolerance upon leptin deficiency,⁹⁹ while the central administration of leptin reversed the pathological metabolic phenotype and caused the inactivation of GSK3 by stimulating the Wnt/LRP6 pathway in NPY neurons,²³⁹ suggesting that the cross-talk of GSK3 and leptin play important roles in the neuroendocrine control of energy homeostasis in the whole body. Therefore, it can be speculated that GSK3 can contribute to the deteriorative effect of hyper- or hypoleptinemia and that normal physiological leptin levels might correct the metabolic phenotype induced by overactivated GSK3.

19 | GSK3 AND ADIPONECTIN

Adiponectin is another adipokine produced by adipocytes. It increases insulin sensitivity, decreases inflammation, improves glucose homeostasis and prevents atherosclerosis, fatty liver disease and obesity. The functional interaction between adiponectin and GSK3 was reported to play important roles in different biological and pathophysiological processes. For example, some studies reported that adiponectin decreased GSK3 inactive phosphorylation and increased its suppressive effect on tumor cell growth.^{240,241} Our studies and those of others demonstrated that inhibiting GSK3 enhanced adiponectin expression in obese adipose tissues,^{85,242} which may act as one of the mechanisms by which GSK3 inhibition improves insulin resistance and ameliorates glucose intolerance caused by obesity. Paradoxically, the constitutive activation of GSK3 caused by a knock-in mutation protected transgenic mice against metabolic syndrome marked by insulin resistance and dyslipidemia. The main mediator of these effects was identified as adiponectin.²⁴³ It is difficult to reconcile these dramatically distinct conclusions based on the currently available evidence. However, glucagon synthesis activity was not affected by mutant GSK3 knock-in, suggesting that mutant GSK3 does not function similarly to wild-type GSK3. Therefore, further studies are needed to understand whether other inactivating modification sites of GSK3 are affected after the known inactive phosphorylation sites are mutated. In addition, the effects generated by the mutant GSK3 might be caused by its interactions with other pathways.

20 | GSK3 AND SIRTUINS

The Sirtuin (Sirt) family proteins, NAD⁺-dependent deacetylases, play many important roles in various tissues, including metabolism-associated tissues such as adipose tissues, skeletal muscle, liver, and brain. Sirt1 can prevent high-fat-induced obesity and metabolic disorders, including glucose intolerance and insulin resistance, which is closely related to the effects of Sirt1 on energy homeostasis through its deacetylation of a range of substrates involved in glucose metabolism, food intake control, insulin signaling, and energy expenditure.^{244–246} These profound effects of Sirts indicate that any factor involved in cross-talk with them may potentially be involved in corresponding cellular processes.

Sirt1 was demonstrated to inactivate GSK3 by activating its upstream kinase, AKT, to increase GSK3 inhibitory phosphorylation during axonogenesis.²⁴⁷ Another Sirt family member, Sirt2, was also found to activate AKT and hence negatively regulate GSK3 activity by promoting its inhibitory Ser9 phosphorylation in AML blasts (Figure 5).²⁴⁸ Sirt2 was also found to inactivate GSK3 β by unknown mechanisms that prohibit retinoic acid-induced ES cell differentiation and lineage commitment.²⁴⁹ These studies suggest that GSK3 may counteract Sirt effects through Sirt-mediated inhibitory phosphorylation of GSK3.

However, GSK3 β activity was also found to be inhibited by acetylation modification in an inhibitory phosphorylation-independent manner at Ser9, and Sirt2 directly interacts and deacetylates GSK3 to derepress acetylation caused inhibition and promote its binding to ATP in the heart. Upon induced Sirt2 deficiency in the mice, increased GSK3 acetylation but not increased Ser9 phosphorylation reduced the activity of GSK3 β . Deacetylated GSK3 then mediated the anti-hypertrophic function of Sirt2 in CMs.²⁵⁰ Another interesting study also reported that Sirt1 derepressed GSK3 via deacetylating it and thus relieved GSK3 β from its inhibition caused by Ser9 phosphorylation. Derepressed GSK3 then stimulated PP1 to dephosphorylate CREB at Ser133 and inhibited CREB, which decreased the synthesis of its target pituitary growth hormones (Figure 5).²⁵¹ While these studies suggest that GSK3 β is regulated by a complicated interplay between different posttranslational modifications, it is difficult to conclude the outcome of Sirt regulation of GSK3. We suspect that the inhibitory effect of Sirts on GSK3, as indicated by inhibitory phosphorylation at the respective serine residue, can be an indirect or byproduct of Sirt functions in the related cellular processes. However, as one type of deacetylases, the derepression of Sirts on GSK3 may be caused directly by increased Sirt deacetylation of GSK3. The effect of Sirts on GSK3 may vary in different tissues and be altered according to changes in physiological conditions. Nevertheless, numerous studies have indicated that GSK3 can negatively influence energy homeostasis and insulin action, while many other studies have

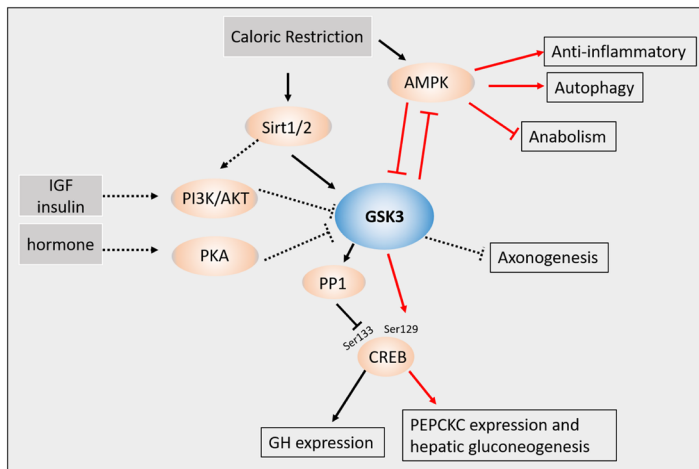


FIGURE 5 GSK3 mediated Sirt and AMPK signaling events. Refer to text for details. Sirt1/2 has dual regulatory effects on GSK3. In response to caloric restriction, Sirt1/2 deacetylates GSK3 competitively activates GSK3 by inhibiting the repressive ser9 phosphorylation of GSK3. Activated GSK3 reduces the Ser133 phosphorylation of CREB by activating its upstream inhibitory kinase PP1, which results in the inactivation of CREB and reduction of anabolic genes such as growth hormone (GH) gene expression (black solid line). Sirts activates GSK3 upstream AKT to increase its inhibitory phosphorylation (dotted line). On the other hand, the caloric restriction also activates AMPK to stimulate GSK3 inhibitory phosphorylation at Ser9. Inactivated GSK3 reduces the phosphorylation of Ser129 of CRE, leading to the inactivation of CREB and the reduction of CREB target gene, PEPCKC, and ultimately, inhibited gluconeogenesis (red line). GSK3 also inhibits AMPK by directly phosphorylating Thr479, resulting in the inhibition of AMPK mediated autophagy and activation of anabolism. AMPK, AMP-activated protein kinase; GSK3, glycogen synthase kinase-3 [Color figure can be viewed at wileyonlinelibrary.com]

demonstrated the beneficial effects of Sirts on energy homeostasis and metabolism. For example, reduced Sirt1 expression and activity but increased GSK3 activity have been observed in specific tissues during chronic inflammation, including obesity-induced adipose tissue with inflammation, brain inflammation caused by neurodegenerative diseases, and arterial inflammation in atherosclerosis.²⁵²

GSK3 β knockout mice are embryonically lethal, suggesting that its function is essential in some respects, thus, it is not surprising to see a beneficial phenotype of GSK3. One possibility is that normal GSK3 levels or activity may function together with Sirt1 to show a beneficial impact, while excess or overactivated GSK3, such as in obese conditions, may exert a harmful influence, especially on homeostasis, which is an outcome in contrast to that promoted by Sirt1.

21 | GSK3 AND AMPK

AMPK, a serine/threonine kinase, is an evolutionarily conserved energy metabolic sensor. AMPK regulates energy homeostasis via modulating lipid and glucose metabolism and insulin sensitivity in multiple tissues. Collectively, these functions endow AMPK with the ability to have beneficial impacts by blocking body weight gain and by attenuating T2D and metabolic syndrome.^{253,254}

The association between GSK3 and AMPK has been observed in many studies. For instance, inhibition of GSK3 is known to indirectly activate AMPK by altering the ATP/AMP ratio in an LKB1-dependent manner and to prevent the initiation of autophagy in prostate cancer cells.¹²⁹ In addition, GSK3 senses anabolic stimuli, such as the insulin signal, which induces its phosphorylation of AMPK and inhibits its activity in multiple cell types, including murine heart tissue and primary hepatocytes; this process is critical for enabling cells to enter an anabolic state in response to environmental cues.²⁵⁵ Surprisingly, GSK3-phosphorylated AMPK was not affected by the inhibitory action of insulin/PI3K-AKT, even though insulin/PI3K pathway activation is required for GSK3 to inactivate AMPK by phosphorylating it on Thr479.²⁵⁵ Although neither the precise molecular mechanism involved nor the implicated physiological significance is known, this novel and reciprocal collaboration between insulin/PI3K and GSK3 on AMPK activity may provide additional evidence indicating that the antidiabetic effect of GSK3 inhibitors *in vitro* and *in vivo* may be mediated by activated AMPK and not exclusively by the regulation of GS activity. In addition, since AMPK acts as an anti-inflammatory factor in many cell populations, inactivation of AMPK by GSK3 also prohibits its anti-inflammatory effect and induces inflammatory responses,²⁵⁶ which is indeed consistent with the study that GSK3 inhibition in obese mice reduced inflammation in adipose tissue and improved inflammation-induced insulin resistance.⁸⁵ It is not clear whether AMPK activity was involved in the effects of GSK3 inhibition on inflammation, but increased AMPK activity may mediate the beneficial outcome of GSK3 inhibition in obese adipose tissue in ways other than GS regulation.

As described above, GSK3 can be regulated by AMPK in response to leptin.^{232,233} In cancer cells, GSK3 is also a potential substrate of AMPK, which is involved in the regulation of cancer cell proliferation and metastasis.²⁵⁷ Horike et al.²⁵⁸ reported that AMPK phosphorylated GSK3 β at Ser9 may result in reduced CREB phosphorylation at Ser129 but not Ser133 and suppressed PEPCK-C gene expression. This reported AMPK-GSK3-CREB-PEPCKC cascade indicates that GSK3 is engaged in anabolic actions, such as gluconeogenesis, by phosphorylating CREB at Ser129 in liver cells (Figure 5). In addition, when activated by Sirt1 through deacetylation under caloric restriction, GSK3 β activates PP1 and dephosphorylates CREB at residue Ser133.²⁵¹ This Sirt1-GSK3-PP1 action of dephosphorylating CREB at Ser133 is important for inhibiting GH synthesis, counteracting the harmful effect of chronic excessive GH, especially under the stress of energy restriction, and facilitating organism adaptation (Figure 5). Insulin and other growth factors, such as IGF-1, can phosphorylate Ser21 in GSK3 α or Ser9 in GSK3 β to inhibit their activity, a process that is mediated by the PI3K-AKT axis. Moreover, in response to hormonal stimulation accompanied by increased cAMP levels, GSK3 is phosphorylated by cAMP-dependent PKA. These observations are consistent with the GSK3 function in suppression of the CREB in mediating the anabolic gene expression.

Finally, GSK3 negatively controlled by AMPK directly affects CREB ser-129 phosphorylation, but GSK3 activated by Sirt1 indirectly influences CREB ser-133 phosphorylation, suggesting that the various substrates of GSK3 lead to different outcomes and render GSK3 a multifunctional enzyme. Overall, different cellular metabolic statuses can lead to preferential GSK3 modifications to induce a unique response to different stimuli through either direct or indirect mechanisms.

22 | GSK3 AND MTOR

The predominant upstream kinase of GSK3 phosphorylation is AKT which is activated by PI3K. Another PI3K dependent GSK3 upstream kinase is ILK, which orchestrates AKT, growth factor, and extracellular matrix signals to inactivate GSK3.²⁵⁹ However, GSK3 activity can also be regulated by other upstream signals independent of AKT. One of the well-known pathways that do not require AKT engagement is the Wnt pathway.²⁶⁰ Another PI3K-AKT independent kinase that phosphorylates GSK is PKA. cAMP-stimulated PKA can phosphorylate and inactivate GSK3.²⁶¹ Under certain conditions, for example, when the stimulation of AKT by amino acids is attenuated or the TSC complex is deficient, GSK3 phosphorylation is regulated by amino acids through nutrient-sensitive mTORC1-S6K1 signaling.^{262,263} mTORC1 is a ubiquitously expressed multimeric protein complex with kinase activity that coordinates to regulate cellular metabolism and cell growth by integrating nutrient (e.g., amino acids) and growth factor signals. Interestingly, the overactivation of mTORC1-S6K1 signaling by excess nutrients, such as amino acids, in the liver and muscle was implicated as a cause of insulin resistance.^{264,265} However, notably, under the conditions of cell-intrinsic insulin resistance, mTORC1 enhances GSK3 inactive phosphorylation. Although it is unknown whether mTORC1 and S6K1 can control GSK3 in insulin-responsive tissues, such as skeletal muscle and liver, this antagonistic relationship between mTORC1 and GSK3 indicates a discrepancy in terms of the effects of both mTORC1 and GSK3 on insulin resistance, despite both mTORC1 and GSK3 being positively associated with insulin resistance in these tissues.^{262,263} Nevertheless, controlling dietary nutrients such as amino acids can control GSK3 activity to affect the metabolic phenotype. Another study reported that mTORC1 suppresses GSK3 to abolish its phosphorylation of Foxk1, a dominant transcriptional factor required for the expression of glycolysis genes and downstream anabolic genes, to increase the expression of Foxk1 target genes and increase glucose metabolism, thus mediating mTORC1-driven metabolic reprogramming and cellular homeostasis.²⁶⁶ On the other hand, some studies demonstrated that GSK3 functions as an upstream kinase of mTORC1 signaling and either negatively or positively regulates mTORC1 activity depending on the signaling molecules that are integrated into the pathway, environmental stimulation, or the experimental conditions. For example, two studies reported that without TSC2 deficiency, TSC2 acts as an intermediary of GSK3 and mTORC1 to modulate GSK3 repression on mTORC1.^{267,268} Similarly, GSK3 β , but not GSK3 α , inhibits mTORC2 activity by phosphorylating its component rictor, leading to AKT inhibition under ER stress to restrain the anabolic effects of the mTORC2-Akt pathway.²¹² Although this conserved regulation of mTORC2-AKT by GSK3 β in vertebrates and invertebrates under ER stress implies that GSK3 β interferes with insulin signaling, it also indicates that GSK3 may contribute to a balance between anabolic and catabolic pathways in response to environmental cues such as ER stress and caloric restriction, which is also indicated by Sirt1-GSK3 axis-regulated GH under caloric restriction. However, when GSK3 phosphorylates the mTOR-associated scaffold protein raptor, the effect of GSK3 on mTORC1 activity is positive.^{269,270} It is still not clearly understood whether these contrasting observations of the relationship between GSK3 and mTOR arise from differences in the cellular context, cell type, environmental cues, or other conditions. But they indeed suggest distinct regulation of GSK3 and mTORC1 and multiple layers of complexity in the regulatory network of mTOR and GSK3. The regulation possibly involves other signal pathways, thus rendering widespread implications for the regulation of cell metabolism, survival, and growth, and for the pathology and treatment of multiple human diseases, such as cancer, diabetes, obesity, and aging.

As we explained above, GSK3 is involved in GH signaling pathways and the energy-sensing system by interacting with Sirts or AMPK. The association revealed between GSK3 and Wnt, PKA and mTOR suggests that GSK3 responds to diverse stimuli, including growth factor (e.g., insulin and IGF-1) cascades or hormonal stimulation (e.g., forskolin) of G protein-coupled receptors. These stimuli may alter intracellular cAMP levels which regulate GSK3 and affect downstream cellular pathways that are implicated in different cellular processes, such as glucose metabolism, cell growth, and cell differentiation. GSK3 activity can also be regulated by modification at other sites, such as Thr43, Ser389, and Thr390 in GSK3 β , which are phosphorylated by Erk and p38 MAPK and correlate with the inactivation of GSK3.^{50,51} Although Tyr279 in GSK3 α or Tyr216 in GSK3 β correlates with the activation of GSK activity,²⁷¹ there are few studies available on this facet of GSK function.

23 | CONCLUDING REMARKS

GSK3 α and GSK3 β appear to play diverse roles in different tissues. GSK3 β is essential for development, and GSK3 α does not compensate for the loss of GSK3 β . In the context of obesity, inflammation, and associated metabolic disorders such as insulin resistance and diabetes, strategies for targeting both GSK3 α and GSK3 β remain promising for the treatment of these diseases. The findings obtained from experiments with GSK3 inhibitors and tissue-specific animal models (including those focused on skeletal muscle, liver, immune cells, and brain) have provided some insights into the treatment of metabolic diseases or other diseases. Despite the recognition of GSK3 being considered a therapeutic target for many diseases, some concerns were also raised owing to the essential roles of GSK3 in some aspects such as in cardiac biology and pathophysiology (discussed in detail in Cardiac Tissue section of this article).⁴³ Another important concern is that inhibiting GSK3 may increase cancer risk because some reports suggest that GSK3 showed a negative association with several tumorigenic. For example, GSK3 inhibition can stabilize β -catenin, a potential proto-oncogene. However, the β -catenin destruction complex assembly is much more sensitive to Axin level than to GSK3 β . The binding of Wnt to the transmembrane Frizzled receptor and its coreceptors, the low-density lipoprotein receptor-related protein -5 or -6 (LRP5/6) leads to the dissociation of the β -catenin destruction complex. GSK3 β is probably not the rate-limiting factor for the Wnt signaling pathway. Activation of the Wnt signaling pathway does not inhibit the enzymatic activity of GSK3, especially the small amount of GSK3 bound to Axin.^{272,273} Instead, Axin protein seems to be the rate-limiting component in regulating the activity of the β -catenin destruction complex at a very low concentration based on experimental data and mathematical models.²⁷⁴ GSK3 β is required at the maximally effective concentration to disrupt β -catenin. The selective deletion of either GSK3 α or β did not affect β -catenin in embryonic stem cells in vitro.³¹ Although the lethality of GSK3 β -null mice limits the investigation of GSK3 β in tumorigenesis, GSK3 α -KO mice did not display an increased propensity for tumorigenesis.¹² β -Catenin activation required silencing of at least three of four GSK3 alleles (>75% inhibition of total GSK3 protein).^{31,136,275} What's more, some Wnt/ β -Catenin degradation pathways are independent of GSK3 activity.²⁷⁶⁻²⁷⁸ One more piece of evidence suggesting that GSK3 inhibition might not lead to oncogenesis is derived from GSK3 inhibitor lithium application to the treatment of BPD; used for several decades, lithium was not found to be associated with increased tumorigenesis or deaths from cancer.¹⁴² A clinical study carried out from over 59,000 patients with this BPD suggests that lithium treatment reduced the cancer risk from 20% to 5%.²⁷⁹ The therapeutic range of lithium is 0.5–1.0 mM²⁸⁰ which causes approximately 20% inhibition of GSK3 and is much lower than the concentration used in cell culture studies (typical lithium concentration is within 10–30 mM for cell culture). Thus, it is reasonable that a weak or moderate inhibition of GSK3, for example to normal physiological levels, wouldn't be expected to induce Wnt signaling toward β -catenin stabilization.

GSK3 is involved in many mechanisms through acting on various substrates and the two isoforms of GSK3 have distinct, albeit not well-defined, functions. Therefore, in addition to partially inhibit total GSK3 activity, developing GSK3 isoform-selective, substrate competitive or disease selective inhibitors could be another feasible strategy. Significant efforts have been made in the past decades to develop potent and highly selective GSK3

isoform-selective inhibitors. Some of them have shown in vitro selectivity and in vivo efficacy in various animal disease models such as AD, cancer, metabolic disorders, etc. Some of them are undergoing preclinical or clinical testing. To date, none of the GSK3 inhibitors has been marketed to clinical application. As an important therapeutic target, it is crucial to identify novel GSK3 inhibitors and to develop precision strategies for individual diseases or patients when targeting GSK3 to achieve the therapeutic outcome without causing unwanted adverse effects.

24 | OUTSTANDING QUESTIONS

What are the specific roles of GSK3 in regulating the metabolic phenotypes in different tissues? How does GSK3 mediate cross-talk between tissues to collectively regulate energy homeostasis and metabolic phenotype?

What are the mechanisms by which GSK3 activity in adipose tissue contributes to obesity and its associated diseases?

What adipokines (such as cytokines and chemokines) in obese adipose tissue require GSK3 for production and for engaging in cross-talk with other tissues in the body for protection against inflammation and insulin resistance?

What are the critical substrates mediating the proinflammatory effects of GSK3 in immune cells?

Does GSK3 in immune cells, in addition to macrophages, also play an important role in regulating the development of inflammation, insulin resistance, and T2D diabetes?

Are direct or newly developed GSK3 inhibitors able to reduce metabolic syndromes, and can they produce beneficial metabolic effects in humans?

Are the protective effects of GSK3 inhibitors in lowering glucose and improving insulin resistance mediated through reduced GSK3-mediated inflammation, in addition to the effects of GSK3 in regulating GS activity?

Do GSK3 inhibitors have protective effects on other metabolic diseases, such as CVD, NAFLD, and atherosclerosis, in addition to insulin resistance and diabetes?

Will the highly selective (isoform, substrate, or disease selective) GSK3 inhibitors help to solve the puzzle associated with the dual function of GSK3 in many disease-related scenarios and therefore be more efficient in achieving the therapeutic effect in treating related diseases? Or would it be more competitive or worthwhile to develop the novel GSK3 inhibitors targeting the GSK3 substrates or even GSK3 regulators?

ACKNOWLEDGMENTS

This work is supported by the Science and Technology Development Fund (FDCT) of Macao SAR to LD (FDCT/0014/2018/A1, FDCT/0117/2018/A3), the Multi-Year Research Grant from the University of Macau to LD (MYRG2018-00158-FHS), and the National Natural Science Foundation of China (NSFC 81772980) to LD.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the corresponding author upon request.

ORCID

Li-jun Di  <https://orcid.org/0000-0002-9558-607X>

REFERENCES

1. Embi N, Rylatt DB, Cohen P. Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase. *Eur J Biochem.* 1980;107(2):519-527.
2. Cline GW, Johnson K, Regittinig W, et al. Effects of a novel glycogen synthase kinase-3 inhibitor on insulin-stimulated glucose metabolism in Zucker diabetic fatty (fa/fa) rats. *Diabetes.* 2002;51(10):2903-2910.
3. Patel S, Doble BW, MacAulay K, Sinclair EM, Drucker DJ, Woodgett JR. Tissue-specific role of glycogen synthase kinase 3beta in glucose homeostasis and insulin action. *Mol Cell Biol.* 2008;28(20):6314-6328.

4. MacAulay K, Hajduch E, Blair AS, Coghlan MP, Smith SA, Hundal HS. Use of lithium and SB-415286 to explore the role of glycogen synthase kinase-3 in the regulation of glucose transport and glycogen synthase. *Eur J Biochem.* 2003;270(18):3829-3838.
5. Ring DB, Johnson KW, Henriksen EJ, et al. Selective glycogen synthase kinase 3 inhibitors potentiate insulin activation of glucose transport and utilization in vitro and in vivo. *Diabetes.* 2003;52(3):588-595.
6. Eldar-Finkelman H. Glycogen synthase kinase-3—a promising therapeutic target: Dr Hagit Eldar-Finkelman interviewed by Emma Quigley. *Expert Opin Ther Targets.* 2006;10(2):199-201.
7. Dokken BB, Sloniger JA, Henriksen EJ. Acute selective glycogen synthase kinase-3 inhibition enhances insulin signaling in prediabetic insulin-resistant rat skeletal muscle. *Am J Physiol Endocrinol Metab.* 2005;288(6):E1188-E1194.
8. Frame S, Zheleva D. Targeting glycogen synthase kinase-3 in insulin signalling. *Expert Opin Ther Targets.* 2006;10(3):429-444.
9. Kaidanovich-Beilin O, Eldar-Finkelman H. Long-term treatment with novel glycogen synthase kinase-3 inhibitor improves glucose homeostasis in ob/ob mice: molecular characterization in liver and muscle. *J Pharmacol Exp Ther.* 2006;316(1):17-24.
10. Ciaraldi TP, Nikolulina SE, Bandukwala RA, Carter L, Henry RR. Role of glycogen synthase kinase-3 alpha in insulin action in cultured human skeletal muscle cells. *Endocrinology.* 2007;148(9):4393-4399.
11. Kerouz NJ, Hörsch D, Pons S, Kahn CR. Differential regulation of insulin receptor substrates-1 and -2 (IRS-1 and IRS-2) and phosphatidylinositol 3-kinase isoforms in liver and muscle of the obese diabetic (ob/ob) mouse. *J Clin Invest.* 1997;100(12):3164-3172.
12. MacAulay K, Doble BW, Patel S, et al. Glycogen synthase kinase 3alpha-specific regulation of murine hepatic glycogen metabolism. *Cell Metab.* 2007;6(4):329-337.
13. Abel ED, Peroni O, Kim JK, et al. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature.* 2001;409(6821):729-733.
14. Eldar-Finkelman H, Krebs EG. Phosphorylation of insulin receptor substrate 1 by glycogen synthase kinase 3 impairs insulin action. *Proc Natl Acad Sci USA.* 1997;94(18):9660-9664.
15. Cormier KW, Woodgett JR. Recent advances in understanding the cellular roles of GSK-3. *F1000Res.* 2017;6:1-7.
16. Robertson H, Hayes JD, Sutherland C. A partnership with the proteasome; the destructive nature of GSK3. *Biochem Pharmacol.* 2018;147:77-92.
17. McCubrey JA, Rakus D, Gizak A, et al. Effects of mutations in Wnt/beta-catenin, hedgehog, Notch and PI3K pathways on GSK-3 activity-Diverse effects on cell growth, metabolism and cancer. *Biochim Biophys Acta.* 2016;1863(12):2942-2976.
18. McCubrey JA, Steelman LS, Bertrand FE, et al. Multifaceted roles of GSK-3 and Wnt/beta-catenin in hematopoiesis and leukemogenesis: opportunities for therapeutic intervention. *Leukemia.* 2014;28(1):15-33.
19. Doble BW, Woodgett JR. GSK-3: tricks of the trade for a multi-tasking kinase. *J Cell Sci.* 2003;116(Pt 7):1175-1186.
20. Grimes CA, Jope RS. The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. *Prog Neurobiol.* 2001;65(4):391-426.
21. Frame S, Cohen P. GSK3 takes centre stage more than 20 years after its discovery. *Biochem J.* 2001;359(Pt 1):1-16.
22. Eldar-Finkelman H. Glycogen synthase kinase 3: an emerging therapeutic target. *Trends Mol Med.* 2002;8(3):126-132.
23. Huang HC, Klein PS. Multiple roles for glycogen synthase kinase-3 as a drug target in Alzheimer's disease. *Curr Drug Targets.* 2006;7(11):1389-97.
24. Schloesser RJ, Huang J, Klein PS, Manji HK. Cellular plasticity cascades in the pathophysiology and treatment of bipolar disorder. *Neuropsychopharmacology.* 2008;33(1):110-133.
25. Muntané G, Dalfó E, Martínez A, Ferrer I. Phosphorylation of tau and alpha-synuclein in synaptic-enriched fractions of the frontal cortex in Alzheimer's disease, and in Parkinson's disease and related alpha-synucleinopathies. *Neuroscience.* 2008;152(4):913-23.
26. Jope RS, Cheng Y, Lowell JA, Worthen RJ, Sitbon YH, Beurel E. Stressed and inflamed, can GSK3 be blamed? *Trends Biochem Sci.* 2017;42(3):180-192.
27. Cohen P, Goedert M. GSK3 inhibitors: development and therapeutic potential. *Nat Rev Drug Discov.* 2004;3(6):479-487.
28. Banerji V, Frumm SM, Ross KN, et al. The intersection of genetic and chemical genomic screens identifies GSK-3alpha as a target in human acute myeloid leukemia. *J Clin Invest.* 2012;122(3):935-947.
29. Woodgett JR. Molecular cloning and expression of glycogen synthase kinase-3/factor A. *EMBO J.* 1990;9(8):2431-2438.
30. Azoulay-Alfaguter I, Yaffe Y, Licht-Murava A, et al. Distinct molecular regulation of glycogen synthase kinase-3alpha isozyme controlled by its N-terminal region: functional role in calcium/calpain signaling. *J Biol Chem.* 2011;286(15):13470-13480.

31. Doble BW, Patel S, Wood GA, Kockeritz LK, Woodgett JR. Functional redundancy of GSK-3alpha and GSK-3beta in Wnt/beta-catenin signaling shown by using an allelic series of embryonic stem cell lines. *Dev Cell*. 2007;12(6):957-971.
32. Kim WY, Zhou FQ, Zhou J, et al. Essential roles for GSK-3s and GSK-3-primed substrates in neurotrophin-induced and hippocampal axon growth. *Neuron*. 2006;52(6):981-996.
33. Zhou FQ, Zhou J, Dedhar S, Wu YH, Snider WD. NGF-induced axon growth is mediated by localized inactivation of GSK-3beta and functions of the microtubule plus end binding protein APC. *Neuron*. 2004;42(6):897-912.
34. Bijur GN, Jope RS. Glycogen synthase kinase-3 beta is highly activated in nuclei and mitochondria. *Neuroreport*. 2003;14(18):2415-2419.
35. Bijur GN, Jope RS. Rapid accumulation of Akt in mitochondria following phosphatidylinositol 3-kinase activation. *J Neurochem*. 2003;87(6):1427-1435.
36. Otori K, Miura T, Tanno M, et al. Ser9 phosphorylation of mitochondrial GSK-3beta is a primary mechanism of cardiomyocyte protection by erythropoietin against oxidant-induced apoptosis. *Am J Physiol Heart Circ Physiol*. 2008;295(5):H2079-H2086.
37. Hetman M, Cavanaugh JE, Kimelman D, Xia Z. Role of glycogen synthase kinase-3beta in neuronal apoptosis induced by trophic withdrawal. *J Neurosci*. 2000;20(7):2567-2574.
38. Nikoulina SE, Ciaraldi TP, Mudaliar S, Mohideen P, Carter L, Henry RR. Potential role of glycogen synthase kinase-3 in skeletal muscle insulin resistance of type 2 diabetes. *Diabetes*. 2000;49(2):263-271.
39. Hoeflich KP, Luo J, Rubie EA, Tsao MS, Jin O, Woodgett JR. Requirement for glycogen synthase kinase-3beta in cell survival and NF-kappaB activation. *Nature*. 2000;406(6791):86-90.
40. Phiel CJ, Wilson CA, Lee VMY, Klein PS. GSK-3alpha regulates production of Alzheimer's disease amyloid-beta peptides. *Nature*. 2003;423(6938):435-439.
41. Zhou J, Freeman TA, Ahmad F, et al. GSK-3alpha is a central regulator of age-related pathologies in mice. *J Clin Invest*. 2013;123(4):1821-1832.
42. Zhou J, Lal H, Chen X, et al. GSK-3alpha directly regulates beta-adrenergic signaling and the response of the heart to hemodynamic stress in mice. *J Clin Invest*. 2010;120(7):2280-2291.
43. Lal H, Ahmad F, Woodgett J, Force T. The GSK-3 family as therapeutic target for myocardial diseases. *Circ Res*. 2015;116(1):138-149.
44. Maurin H, Lechat B, Dewachter I, et al. Neurological characterization of mice deficient in GSK3alpha highlight pleiotropic physiological functions in cognition and pathological activity as Tau kinase. *Mol Brain*. 2013;6:27.
45. Bhattacharjee R, Goswami S, Dudiki T, et al. Targeted disruption of glycogen synthase kinase 3A (GSK3A) in mice affects sperm motility resulting in male infertility. *Biol Reprod*. 2015;92(3):65.
46. Bhattacharjee R, Goswami S, Dey S, et al. Isoform-specific requirement for GSK3alpha in sperm for male fertility. *Biol Reprod*. 2018;99(2):384-394.
47. Cervello M, Augello G, Cusimano A, et al. Pivotal roles of glycogen synthase-3 in hepatocellular carcinoma. *Adv Biol Regul*. 2017;65:59-76.
48. Domoto T, Pyko IV, Furuta T, et al. Glycogen synthase kinase-3beta is a pivotal mediator of cancer invasion and resistance to therapy. *Cancer Sci*. 2016;107(10):1363-1372.
49. Hernández F, Langa E, Cuadros R, Avila J, Villanueva N. Regulation of GSK3 isoforms by phosphatases PP1 and PP2A. *Mol Cell Biochem*. 2010;344(1-2):211-215.
50. Thornton TM, Pedraza-Alva G, Deng B, et al. Phosphorylation by p38 MAPK as an alternative pathway for GSK3beta inactivation. *Science*. 2008;320(5876):667-670.
51. Ding Q, Xia W, Liu JC, et al. Erk associates with and primes GSK-3beta for its inactivation resulting in upregulation of beta-catenin. *Mol Cell*. 2005;19(2):159-170.
52. Lochhead PA, Kinstrie R, Sibbet G, Rawjee T, Morrice N, Cleghon V. A chaperone-dependent GSK3beta transitional intermediate mediates activation-loop autophosphorylation. *Mol Cell*. 2006;24(4):627-633.
53. Jope RS, Yuskaitis CJ, Beurel E. Glycogen synthase kinase-3 (GSK3): inflammation, diseases, and therapeutics. *Neurochem Res*. 2007;32(4-5):577-595.
54. McManus EJ, Sakamoto K, Armit LJ, et al. Role that phosphorylation of GSK3 plays in insulin and Wnt signalling defined by knockin analysis. *EMBO J*. 2005;24(8):1571-1583.
55. Ciaraldi TP, Oh DK, Christiansen L, et al. Tissue-specific expression and regulation of GSK-3 in human skeletal muscle and adipose tissue. *Am J Physiol Endocrinol Metab*. 2006;291(5):E891-E898.
56. Eldar-Finkelman H, Schreyer SA, Shinohara MM, LeBoeuf RC, Krebs EG. Increased glycogen synthase kinase-3 activity in diabetes- and obesity-prone C57BL/6J mice. *Diabetes*. 1999;48(8):1662-1666.
57. Pearce NJ, Arch JR, Clapham JC, et al. Development of glucose intolerance in male transgenic mice overexpressing human glycogen synthase kinase-3beta on a muscle-specific promoter. *Metabolism*. 2004;53(10):1322-1330.

58. Patel S, Macaulay K, Woodgett JR. Tissue-specific analysis of glycogen synthase kinase-3alpha (GSK-3alpha) in glucose metabolism: effect of strain variation. *PLoS One*. 2011;6(1):e15845.
59. Lee J, Kim MS. The role of GSK3 in glucose homeostasis and the development of insulin resistance. *Diabetes Res Clin Pract*. 2007;77(Suppl 1):S49-S57.
60. Nikoulina SE, Ciaraldi TP, Mudaliar S, Carter L, Johnson K, Henry RR. Inhibition of glycogen synthase kinase 3 improves insulin action and glucose metabolism in human skeletal muscle. *Diabetes*. 2002;51(7):2190-2198.
61. Ma Z, Zhong Z, Zheng Z, Shi XM, Zhang W. Inhibition of glycogen synthase kinase-3beta attenuates glucocorticoid-induced suppression of myogenic differentiation in vitro. *PLoS One*. 2014;9(8):e105528.
62. van der Velden J, Schols AMWJ, Willems J, Kelders MCJM, Langen RCJ. Glycogen synthase kinase 3 suppresses myogenic differentiation through negative regulation of NFATc3. *J Biol Chem*. 2008;283(1):358-366.
63. van der Velden JL, Langen RC, Kelders MC, Wouters EF, Janssen-Heininger YM, Schols AM. Inhibition of glycogen synthase kinase-3beta activity is sufficient to stimulate myogenic differentiation. *Am J Physiol Cell Physiol*. 2006;290(2):C453-C462.
64. Wei C, Stock L, Valanejad L, et al. Correction of GSK3beta at young age prevents muscle pathology in mice with myotonic dystrophy type 1. *FASEB J*. 2018;32(4):2073-2085.
65. Rochat A, Fernandez A, Vandromme M, et al. Insulin and wnt1 pathways cooperate to induce reserve cell activation in differentiation and myotube hypertrophy. *Mol Biol Cell*. 2004;15(10):4544-4555.
66. Pansters NA, van der Velden JL, Kelders MC, Laeremans H, Schols AM, Langen RC. Segregation of myoblast fusion and muscle-specific gene expression by distinct ligand-dependent inactivation of GSK-3beta. *Cell Mol Life Sci*. 2011;68(3):523-535.
67. Verhees KJ, Schols AM, Kelders MC, Op den Kamp CM, van der Velden JL, Langen RC. Glycogen synthase kinase-3beta is required for the induction of skeletal muscle atrophy. *Am J Physiol Cell Physiol*. 2011;301(5):C995-C1007.
68. Verhees KJ, Pansters NA, Baarsma HA, et al. Pharmacological inhibition of GSK-3 in a guinea pig model of LPS-induced pulmonary inflammation: II. Effects on skeletal muscle atrophy. *Respir Res*. 2013;14:117.
69. Pansters NA, Schols AM, Verhees KJ, et al. Muscle-specific GSK-3beta ablation accelerates regeneration of disuse-atrophied skeletal muscle. *Biochim Biophys Acta*. 2015;1852(3):490-506.
70. Markuns JF, Wojtaszewski JF, Goodyear LJ. Insulin and exercise decrease glycogen synthase kinase-3 activity by different mechanisms in rat skeletal muscle. *J Biol Chem*. 1999;274(35):24896-24900.
71. Theeuwes WF, Gosker HR, Langen R, Pansters N, Schols A, Remels A. Inactivation of glycogen synthase kinase 3beta (GSK-3beta) enhances mitochondrial biogenesis during myogenesis. *Biochim Biophys Acta, Mol Basis Dis*. 2018;1864(9 Pt B):2913-2926.
72. Lochhead PA, Coghlan M, Rice SQ, Sutherland C. Inhibition of GSK-3 selectively reduces glucose-6-phosphatase and phosphatase and phosphoenolpyruvate carboxykinase gene expression. *Diabetes*. 2001;50(5):937-946.
73. Moule SK, Welsh GI, Edgell NJ, Foulstone EJ, Proud CG, Denton RM. Regulation of protein kinase B and glycogen synthase kinase-3 by insulin and beta-adrenergic agonists in rat epididymal fat cells. Activation of protein kinase B by wortmannin-sensitive and -insensitive mechanisms. *J Biol Chem*. 1997;272(12):7713-7719.
74. Brady MJ, Bourbonais FJ, Saltiel AR. The activation of glycogen synthase by insulin switches from kinase inhibition to phosphatase activation during adipogenesis in 3T3-L1 cells. *J Biol Chem*. 1998;273(23):14063-14066.
75. Wang L, Wang Y, Meng Y, Zhang C, Di L. GSK3-activated STAT5 regulates expression of SFRPs to modulate adipogenesis. *FASEB J*. 2018;32:fj201701314R-fj2017014726.
76. Ross SE, Hemati N, Longo KA, et al. Inhibition of adipogenesis by Wnt signaling. *Science*. 2000;289(5481):950-953.
77. Ross SE, Erickson RL, Hemati N, MacDougald OA. Glycogen synthase kinase 3 is an insulin-regulated C/EBPalpha kinase. *Mol Cell Biol*. 1999;19(12):8433-8441.
78. Wang L, Di LJ. Wnt/beta-catenin mediates AICAR effect to increase GATA3 expression and inhibit adipogenesis. *J Biol Chem*. 2015;290(32):19458-19468.
79. Kennell JA, MacDougald OA. Wnt signaling inhibits adipogenesis through beta-catenin-dependent and -independent mechanisms. *J Biol Chem*. 2005;280(25):24004-24010.
80. Kanazawa A, Tsukada S, Kamiyama M, Yanagimoto T, Nakajima M, Maeda S. Wnt5b partially inhibits canonical Wnt/beta-catenin signaling pathway and promotes adipogenesis in 3T3-L1 preadipocytes. *Biochem Biophys Res Commun*. 2005;330(2):505-510.
81. Zaragosi LE, Wdziekonski B, Fontaine C, Villageois P, Peraldi P, Dani C. Effects of GSK3 inhibitors on in vitro expansion and differentiation of human adipose-derived stem cells into adipocytes. *BMC Cell Biol*. 2008;9:11.
82. Song WJ, Song EA, Jung MS, et al. Phosphorylation and inactivation of glycogen synthase kinase 3beta (GSK3beta) by dual-specificity tyrosine phosphorylation-regulated kinase 1A (Dyrk1A). *J Biol Chem*. 2015;290(4):2321-2333.
83. Markussen LK, Winther S, Wicksteed B, Hansen JB. GSK3 is a negative regulator of the thermogenic program in brown adipocytes. *Sci Rep*. 2018;8(1):3469.

84. Lappas M. GSK3beta is increased in adipose tissue and skeletal muscle from women with gestational diabetes where it regulates the inflammatory response. *PLoS One*. 2014;9(12):e115854.
85. Wang L, Wang Y, Zhang C, et al. Inhibiting glycogen synthase kinase 3 reverses obesity-induced white adipose tissue inflammation by regulating apoptosis inhibitor of macrophage/CD5L-mediated macrophage migration. *Arterioscler Thromb Vasc Biol*. 2018;38(9):2103-2116.
86. Mussmann R, Geese M, Harder F, et al. Inhibition of GSK3 promotes replication and survival of pancreatic beta cells. *J Biol Chem*. 2007;282(16):12030-12037.
87. Srinivasan S, Ohsugi M, Liu Z, Fatrai S, Bernal-Mizrachi E, Permutt MA. Endoplasmic reticulum stress-induced apoptosis is partly mediated by reduced insulin signaling through phosphatidylinositol 3-kinase/Akt and increased glycogen synthase kinase-3beta in mouse insulinoma cells. *Diabetes*. 2005;54(4):968-975.
88. Tanabe K, Liu Z, Patel S, et al. Genetic deficiency of glycogen synthase kinase-3beta corrects diabetes in mouse models of insulin resistance. *PLoS Biol*. 2008;6(2):e37.
89. Liu Y, Tanabe K, Baronnier D, et al. Conditional ablation of Gsk-3beta in islet beta cells results in expanded mass and resistance to fat feeding-induced diabetes in mice. *Diabetologia*. 2010;53(12):2600-2610.
90. Liu Z, Tanabe K, Bernal-Mizrachi E, Permutt MA. Mice with beta cell overexpression of glycogen synthase kinase-3beta have reduced beta cell mass and proliferation. *Diabetologia*. 2008;51(4):623-631.
91. Boucher MJ, Selander L, Carlsson L, Edlund H. Phosphorylation marks IPF1/PDX1 protein for degradation by glycogen synthase kinase 3-dependent mechanisms. *J Biol Chem*. 2006;281(10):6395-6403.
92. Song L, De Sarno P, Jope RS. Central role of glycogen synthase kinase-3beta in endoplasmic reticulum stress-induced caspase-3 activation. *J Biol Chem*. 2002;277(47):44701-44708.
93. Shen W, Taylor B, Jin Q, et al. Inhibition of DYRK1A and GSK3B induces human beta-cell proliferation. *Nat Commun*. 2015;6:8372.
94. Könnner AC, Janoschek R, Plum L, et al. Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production. *Cell Metab*. 2007;5(6):438-449.
95. Pocai A, Obici S, Schwartz GJ, Rossetti L. A brain-liver circuit regulates glucose homeostasis. *Cell Metab*. 2005;1(1):53-61.
96. Obici S, Zhang BB, Karknias G, Rossetti L. Hypothalamic insulin signaling is required for inhibition of glucose production. *Nat Med*. 2002;8(12):1376-1382.
97. Yao HB, Shaw PC, Wong CC, Wan DC. Expression of glycogen synthase kinase-3 isoforms in mouse tissues and their transcription in the brain. *J Chem Neuroanat*. 2002;23(4):291-297.
98. Jolivald CG, Lee CA, Beiswenger KK, et al. Defective insulin signaling pathway and increased glycogen synthase kinase-3 activity in the brain of diabetic mice: parallels with Alzheimer's disease and correction by insulin. *J Neurosci Res*. 2008;86(15):3265-3274.
99. Benzler J, Ganjam GK, Krüger M, et al. Hypothalamic glycogen synthase kinase 3beta has a central role in the regulation of food intake and glucose metabolism. *Biochem J*. 2012;447(1):175-184.
100. Martin SA, Souder DC, Miller KN, et al. GSK3beta regulates brain energy metabolism. *Cell Rep*. 2018;23(7):1922-1931.
101. Gimenez-Cassina A, Lim F, Cerrato T, Palomo GM, Diaz-Nido J. Mitochondrial hexokinase II promotes neuronal survival and acts downstream of glycogen synthase kinase-3. *J Biol Chem*. 2009;284(5):3001-3011.
102. Wang Y, Chan SL, Miele L, et al. Involvement of Notch signaling in hippocampal synaptic plasticity. *Proc Natl Acad Sci USA*. 2004;101(25):9458-9462.
103. Beaulieu JM, Sotnikova TD, Marion S, Lefkowitz RJ, Gainetdinov RR, Caron MG. An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior. *Cell*. 2005;122(2):261-273.
104. Mao Y, Ge X, Frank CL, et al. Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3beta/beta-catenin signaling. *Cell*. 2009;136(6):1017-1031.
105. Beaulieu JM, Gainetdinov RR, Caron MG. Akt/GSK3 signaling in the action of psychotropic drugs. *Annu Rev Pharmacol Toxicol*. 2009;49:327-347.
106. Yoshimura T, Kawano Y, Arimura N, Kawabata S, Kikuchi A, Kaibuchi K. GSK-3beta regulates phosphorylation of CRMP-2 and neuronal polarity. *Cell*. 2005;120(1):137-149.
107. Zhou FQ, Snider WD. Cell biology. GSK-3beta and microtubule assembly in axons. *Science*. 2005;308(5719):211-214.
108. Haq S, Choukroun G, Kang ZB, et al. Glycogen synthase kinase-3beta is a negative regulator of cardiomyocyte hypertrophy. *J Cell Biol*. 2000;151(1):117-130.
109. Morisco C, Zebrowski D, Condorelli G, Tschlis P, Vatner SF, Sadoshima J. The Akt-glycogen synthase kinase 3beta pathway regulates transcription of atrial natriuretic factor induced by beta-adrenergic receptor stimulation in cardiac myocytes. *J Biol Chem*. 2000;275(19):14466-14475.

110. Matsuda T, Zhai P, Maejima Y, et al. Distinct roles of GSK-3 α and GSK-3 β phosphorylation in the heart under pressure overload. *Proc Natl Acad Sci USA*. 2008;105(52):20900-20905.
111. Lal H, Zhou J, Ahmad F, et al. Glycogen synthase kinase-3 α limits ischemic injury, cardiac rupture, post-myocardial infarction remodeling and death. *Circulation*. 2012;125(1):65-75.
112. Hirofumi S, Zhai P, Tomita H, et al. Inhibition of glycogen synthase kinase 3 β during heart failure is protective. *Circ Res*. 2007;101(11):1164-1174.
113. Woulfe KC, Gao E, Lal H, et al. Glycogen synthase kinase-3 β regulates post-myocardial infarction remodeling and stress-induced cardiomyocyte proliferation in vivo. *Circ Res*. 2010;106(10):1635-1645.
114. Mora A, Sakamoto K, McManus EJ, Alessi DR. Role of the PDK1-PKB-GSK3 pathway in regulating glycogen synthase and glucose uptake in the heart. *FEBS Lett*. 2005;579(17):3632-3638.
115. Omar MA, Wang L, Clanachan AS. Cardioprotection by GSK-3 inhibition: role of enhanced glycogen synthesis and attenuation of calcium overload. *Cardiovasc Res*. 2010;86(3):478-486.
116. Gupte M, Tumurluru S, Sui JY, et al. Cardiomyocyte-specific deletion of GSK-3 β leads to cardiac dysfunction in a diet induced obesity model. *Int J Cardiol*. 2018;259:145-152.
117. Gupte M, Umbarkar P, Singh AP, Zhang Q, Tousif S, Lal H. Deletion of cardiomyocyte glycogen synthase kinase-3 β (GSK-3 β) improves systemic glucose tolerance with maintained heart function in established obesity. *Cells*. 2020;9(5):1120.
118. Flepisi TB, Lochner A, Huisamen B. The consequences of long-term glycogen synthase kinase-3 inhibition on normal and insulin resistant rat hearts. *Cardiovasc Drugs Ther*. 2013;27(5):381-392.
119. Wang Y, Feng W, Xue W, et al. Inactivation of GSK-3 β by metallothionein prevents diabetes-related changes in cardiac energy metabolism, inflammation, nitrosative damage, and remodeling. *Diabetes*. 2009;58(6):1391-1402.
120. Luo J. Glycogen synthase kinase 3 β (GSK3 β) in tumorigenesis and cancer chemotherapy. *Cancer Lett*. 2009;273(2):194-200.
121. Shakoori A, Ougolkov A, Yu ZW, et al. Deregulated GSK3 β activity in colorectal cancer: its association with tumor cell survival and proliferation. *Biochem Biophys Res Commun*. 2005;334(4):1365-1373.
122. Ougolkov AV, Fernandez-Zapico ME, Savoy DN, Urrutia RA, Billadeau DD. Glycogen synthase kinase-3 β participates in nuclear factor kappaB-mediated gene transcription and cell survival in pancreatic cancer cells. *Cancer Res*. 2005;65(6):2076-2081.
123. Zhou W, Wang L, Gou SM, et al. ShRNA silencing glycogen synthase kinase-3 β inhibits tumor growth and angiogenesis in pancreatic cancer. *Cancer Lett*. 2012;316(2):178-186.
124. Ougolkov AV, Billadeau DD. Targeting GSK-3: a promising approach for cancer therapy? *Future Oncol*. 2006;2(1):91-100.
125. Farago M, Dominguez I, Landesman-Bollag E, et al. Kinase-inactive glycogen synthase kinase 3 β promotes Wnt signaling and mammary tumorigenesis. *Cancer Res*. 2005;65(13):5792-5801.
126. Dal Col J, Dolcetti R. GSK-3 β inhibition: at the crossroad between Akt and mTOR constitutive activation to enhance cyclin D1 protein stability in mantle cell lymphoma. *Cell Cycle*. 2008;7(18):2813-2816.
127. Elgendy M, Cirò M, Hosseini A, et al. Combination of hypoglycemia and metformin impairs tumor metabolic plasticity and growth by modulating the PP2A-GSK3 β -MCL-1 Axis. *Cancer Cell*. 2019;35(5):798-815.
128. Pal K, Cao Y, Gaisina IN, et al. Inhibition of GSK-3 induces differentiation and impaired glucose metabolism in renal cancer. *Mol Cancer Ther*. 2014;13(2):285-296.
129. Sun A, Li C, Chen R, et al. GSK-3 β controls autophagy by modulating LKB1-AMPK pathway in prostate cancer cells. *Prostate*. 2016;76(2):172-183.
130. Azoulay-Alfaguter I, Elya R, Avrahami L, Katz A, Eldar-Finkelman H. Combined regulation of mTORC1 and lysosomal acidification by GSK-3 suppresses autophagy and contributes to cancer cell growth. *Oncogene*. 2015;34(35):4613-4623.
131. Marchand B, Arsenaud D, Raymond-Fleury A, Boisvert FM, Boucher MJ. Glycogen synthase kinase-3 (GSK3) inhibition induces prosurvival autophagic signals in human pancreatic cancer cells. *J Biol Chem*. 2015;290(9):5592-5605.
132. Rosenfeldt MT, O'Prey J, Morton JP, et al. p53 status determines the role of autophagy in pancreatic tumour development. *Nature*. 2013;504(7479):296-300.
133. Lin SY, Li TY, Liu Q, et al. GSK3-TIP60-ULK1 signaling pathway links growth factor deprivation to autophagy. *Science*. 2012;336(6080):477-481.
134. Jellusova J, Cato MH, Apgar JR, et al. Gsk3 is a metabolic checkpoint regulator in B cells. *Nat Immunol*. 2017;18(3):303-312.
135. Momcilovic M, Bailey ST, Lee JT, et al. The GSK3 signaling axis regulates adaptive glutamine metabolism in lung squamous cell carcinoma. *Cancer Cell*. 2018;33(5):905-921.

136. Beurel E, Grieco SF, Jope RS. Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. *Pharmacol Ther.* 2015;148:114-131.
137. Augello G, Emma MR, Cusimano A, et al. The Role of GSK-3 in Cancer Immunotherapy: GSK-3 inhibitors as a new frontier in cancer treatment. *Cells.* 2020;9(6):1427.
138. Pandey MK, DeGrado TR. Glycogen synthase kinase-3 (GSK-3)-targeted therapy and imaging. *Theranostics.* 2016; 6(4):571-593.
139. Palomo V, Martinez A. Glycogen synthase kinase 3 (GSK-3) inhibitors: a patent update (2014-2015). *Expert Opin Ther Pat.* 2017;27(6):657-666.
140. Roca C, Campillo NE. Glycogen synthase kinase 3 (GSK-3) inhibitors: a patent update (2016-2019). *Expert Opin Ther Pat.* 2020;30(11):863-872.
141. Maqbool M, Hoda N. GSK3 inhibitors in the therapeutic development of diabetes, cancer and neurodegeneration: past, present and future. *Curr Pharm Des.* 2017;23(29):4332-4350.
142. Eldar-Finkelman H, Martinez A. GSK-3 inhibitors: preclinical and clinical focus on CNS. *Front Mol Neurosci.* 2011;4: 32.
143. Maqbool M, Mobashir M, Hoda N. Pivotal role of glycogen synthase kinase-3: a therapeutic target for Alzheimer's disease. *Eur J Med Chem.* 2016;107:63-81.
144. Lei P, Aytton S, Bush AI, Adlard PA. GSK-3 in neurodegenerative diseases. *Int J Alzheimers Dis.* 2011;2011:189246.
145. Lo Monte F, Kramer T, Gu J, et al. Structure-based optimization of oxadiazole-based GSK-3 inhibitors. *Eur J Med Chem.* 2013;61:26-40.
146. Licht-Murava A, Eldar-Finkelman H. Exploiting substrate recognition for selective inhibition of protein kinases. *Curr Pharm Des.* 2012;18(20):2914-2920.
147. Noori MS, Bhatt PM, Courreges MC, et al. Identification of a novel selective and potent inhibitor of glycogen synthase kinase-3. *Am J Physiol Cell Physiol.* 2019;317(6):C1289-C1303.
148. McCamphill PK, Stoppel LJ, Senter RK, et al. Selective inhibition of glycogen synthase kinase 3alpha corrects pathophysiology in a mouse model of fragile X syndrome. *Sci Transl Med.* 2020;12(544):eaam8572.
149. Wagner FF, Benajiba L, Campbell AJ, et al. Exploiting an Asp-Glu "switch" in glycogen synthase kinase 3 to design paralog-selective inhibitors for use in acute myeloid leukemia. *Sci Transl Med.* 2018;10(431):eaam8460.
150. Saraswati AP, Ali Hussaini SM, Krishna NH, Babu BN, Kamal A. Glycogen synthase kinase-3 and its inhibitors: potential target for various therapeutic conditions. *Eur J Med Chem.* 2018;144:843-858.
151. Hoshi M, Sato M, Matsumoto S, et al. Spherical aggregates of beta-amyloid (amylospheroid) show high neurotoxicity and activate tau protein kinase I/glycogen synthase kinase-3beta. *Proc Natl Acad Sci USA.* 2003;100(11):6370-6375.
152. del Ser T, Steinwachs KC, Gertz HJ, et al. Treatment of Alzheimer's disease with the GSK-3 inhibitor tideglusib: a pilot study. *J Alzheimers Dis.* 2013;33(1):205-215.
153. Duda P, Akula SM, Abrams SL, et al. Targeting GSK3 and associated signaling pathways involved in cancer. *Cells.* 2020;9(5):1110.
154. Polakis P. The oncogenic activation of beta-catenin. *Curr Opin Genet Dev.* 1999;9(1):15-21.
155. Ueda M, Stefan T, Stetson L, et al. Phase I trial of lithium and tretinoin for treatment of relapsed and refractory non-promyelocytic acute myeloid leukemia. *Front Oncol.* 2020;10:327.
156. Gray JE, Infante JR, Brail LH, et al. A first-in-human phase I dose-escalation, pharmacokinetic, and pharmacodynamic evaluation of intravenous LY2090314, a glycogen synthase kinase 3 inhibitor, administered in combination with pemetrexed and carboplatin. *Invest New Drugs.* 2015;33(6):1187-1196.
157. Sfera A, Osorio C, Inderias LA, Parker V, Price AI, Cummings M. The obesity-impulsivity axis: potential metabolic interventions in chronic psychiatric patients. *Front Psychiatry.* 2017;8:20.
158. Haupt DW, Newcomer JW. Abnormalities in glucose regulation associated with mental illness and treatment. *J Psychosom Res.* 2002;53(4):925-933.
159. St-Pierre J, Lin J, Krauss S, et al. Bioenergetic analysis of peroxisome proliferator-activated receptor gamma coactivators 1alpha and 1beta (PGC-1alpha and PGC-1beta) in muscle cells. *J Biol Chem.* 2003;278(29):26597-26603.
160. Lehman JJ, Barger PM, Kovacs A, Saffitz JE, Medeiros DM, Kelly DP. Peroxisome proliferator-activated receptor gamma coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest.* 2000;106(7):847-856.
161. Chau MD, Gao J, Yang Q, Wu Z, Gromada J. Fibroblast growth factor 21 regulates energy metabolism by activating the AMPK-SIRT1-PGC-1alpha pathway. *Proc Natl Acad Sci USA.* 2010;107(28):12553-12558.
162. Lin J, Wu H, Tarr PT, et al. Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. *Nature.* 2002;418(6899):797-801.
163. Puigserver P, Rhee J, Donovan J, et al. Insulin-regulated hepatic gluconeogenesis through FOXO1-PGC-1alpha interaction. *Nature.* 2003;423(6939):550-555.
164. Yoon JC, Puigserver P, Chen G, et al. Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature.* 2001;413(6852):131-138.

165. Vega RB, Huss JM, Kelly DP. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol Cell Biol.* 2000;20(5):1868-1876.
166. Wu Z, Puigserver P, Andersson U, et al. Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell.* 1999;98(1):115-124.
167. Canto C, Auwerx J. PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Curr Opin Lipidol.* 2009;20(2):98-105.
168. Olson BL, Hock MB, Ekholm-Reed S, et al. SCFCdc4 acts antagonistically to the PGC-1alpha transcriptional coactivator by targeting it for ubiquitin-mediated proteolysis. *Genes Dev.* 2008;22(2):252-264.
169. Anderson RM, Barger JL, Edwards MG, et al. Dynamic regulation of PGC-1alpha localization and turnover implicates mitochondrial adaptation in calorie restriction and the stress response. *Aging cell.* 2008;7(1):101-111.
170. Undi RB, Gutti U, Gutti RK. LiCl regulates mitochondrial biogenesis during megakaryocyte development. *J Trace Elem Med Biol.* 2017;39:193-201.
171. Xu R, Hu Q, Ma Q, Liu C, Wang G. The protease Omi regulates mitochondrial biogenesis through the GSK3beta/PGC-1alpha pathway. *Cell Death Dis.* 2014;5:e1373.
172. Yang K, Chen Z, Gao J, et al. The key roles of GSK-3beta in regulating mitochondrial activity. *Cell Physiol Biochem.* 2017;44(4):1445-1459.
173. Llorens-Martín M, López-Doménech G, Soriano E, Avila J. GSK3beta is involved in the relief of mitochondria pausing in a Tau-dependent manner. *PLoS One.* 2011;6(11):e27686.
174. Hollenbeck PJ, Saxton WM. The axonal transport of mitochondria. *J Cell Sci.* 2005;118(Pt 23):5411-5419.
175. Ogawa F, Murphy LC, Malavasi EL, et al. NDE1 and GSK3beta associate with TRAK1 and regulate axonal mitochondrial motility: identification of cyclic AMP as a novel modulator of axonal mitochondrial trafficking. *ACS Chem Neurosci.* 2016;7(5):553-564.
176. Chen S, Owens GC, Crossin KL, Edelman DB. Serotonin stimulates mitochondrial transport in hippocampal neurons. *Mol Cell Neurosci.* 2007;36(4):472-483.
177. Chen S, Owens GC, Makarenkova H, Edelman DB. HDAC6 regulates mitochondrial transport in hippocampal neurons. *PLoS One.* 2010;5(5):e10848.
178. Hoshi M, Sato M, Kondo S, et al. Different localization of tau protein kinase I/glycogen synthase kinase-3 beta from glycogen synthase kinase-3 alpha in cerebellum mitochondria. *J Biochem.* 1995;118(4):683-685.
179. Hoshi M, Takashima A, Noguchi K, et al. Regulation of mitochondrial pyruvate dehydrogenase activity by tau protein kinase I/glycogen synthase kinase 3beta in brain. *Proc Natl Acad Sci USA.* 1996;93(7):2719-2723.
180. Nishihara M, Miura T, Miki T, et al. Modulation of the mitochondrial permeability transition pore complex in GSK-3beta-mediated myocardial protection. *J Mol Cell Cardiol.* 2007;43(5):564-570.
181. Juhaszova M, Zorov DB, Kim SH, et al. Glycogen synthase kinase-3beta mediates convergence of protection signaling to inhibit the mitochondrial permeability transition pore. *J Clin Invest.* 2004;113(11):1535-1549.
182. Vadrot N, Ghanem S, Braut F, et al. Mitochondrial DNA maintenance is regulated in human hepatoma cells by glycogen synthase kinase 3beta and p53 in response to tumor necrosis factor alpha. *PLoS One.* 2012;7(7):e40879.
183. King TD, Clodfelder-Miller B, Barksdale KA, Bijur GN. Unregulated mitochondrial GSK3beta activity results in NADH: ubiquinone oxidoreductase deficiency. *Neurotox Res.* 2008;14(4):367-382.
184. Chiara F, Rasola A. GSK-3 and mitochondria in cancer cells. *Front Oncol.* 2013;3:16.
185. Petit-Paitel A, Brau F, Cazareth J, Chabry J. Involvement of cytosolic and mitochondrial GSK-3beta in mitochondrial dysfunction and neuronal cell death of MPTP/MPP-treated neurons. *PLoS One.* 2009;4(5):e5491.
186. Zhang YJ, Xu YF, Liu YH, Yin J, Wang JZ. Nitric oxide induces tau hyperphosphorylation via glycogen synthase kinase-3beta activation. *FEBS Lett.* 2005;579(27):6230-6236.
187. Flügel D, Görlach A, Michiels C, Kietzmann T. Glycogen synthase kinase 3 phosphorylates hypoxia-inducible factor 1alpha and mediates its destabilization in a VHL-independent manner. *Mol Cell Biol.* 2007;27(9):3253-3265.
188. Mottet D, Dumont V, Deccache Y, et al. Regulation of hypoxia-inducible factor-1alpha protein level during hypoxic conditions by the phosphatidylinositol 3-kinase/Akt/glycogen synthase kinase 3beta pathway in HepG2 cells. *J Biol Chem.* 2003;278(33):31277-31285.
189. Flügel D, Gölach A, Kietzmann T. GSK-3beta regulates cell growth, migration, and angiogenesis via Fbw7 and USP28-dependent degradation of HIF-1alpha. *Blood.* 2012;119(5):1292-1301.
190. Cassavaugh JM, Hale SA, Wellman TL, Howe AK, Wong C, Lounsbury KM. Negative regulation of HIF-1alpha by an FBW7-mediated degradation pathway during hypoxia. *J Cell Biochem.* 2011;112(12):3882-3890.
191. Schnitzer SE, Schmid T, Zhou J, Eisenbrand G, Brüne B. Inhibition of GSK3beta by indirubins restores HIF-1alpha accumulation under prolonged periods of hypoxia/anoxia. *FEBS Lett.* 2005;579(2):529-533.

192. Loberg RD, Vesely E, Brosius FC 3rd. Enhanced glycogen synthase kinase-3 β activity mediates hypoxia-induced apoptosis of vascular smooth muscle cells and is prevented by glucose transport and metabolism. *J Biol Chem*. 2002; 277(44):41667-41673.
193. Roh MS, Eom TY, Zmijewska AA, De Sarno P, Roth KA, Jope RS. Hypoxia activates glycogen synthase kinase-3 in mouse brain in vivo: protection by mood stabilizers and imipramine. *Biol Psychiatry*. 2005;57(3):278-286.
194. Goldbart A, Cheng ZJ, Brittan KR, Gozal D. Intermittent hypoxia induces time-dependent changes in the protein kinase B signaling pathway in the hippocampal CA1 region of the rat. *Neurobiol Dis*. 2003;14(3):440-446.
195. Beitner-Johnson D, Rust RT, Hsieh TC, Millhorn DE. Hypoxia activates Akt and induces phosphorylation of GSK-3 in PC12 cells. *Cell Signal*. 2001;13(1):23-27.
196. Chen EY, Mazure NM, Cooper JA, Giaccia AJ. Hypoxia activates a platelet-derived growth factor receptor/phosphatidylinositol 3-kinase/Akt pathway that results in glycogen synthase kinase-3 inactivation. *Cancer Res*. 2001; 61(6):2429-2433.
197. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiol Rev*. 2014;94(3):909-950.
198. Waypa GB, Marks JD, Guzy RD, et al. Superoxide generated at mitochondrial complex III triggers acute responses to hypoxia in the pulmonary circulation. *Am J Respir Crit Care Med*. 2013;187(4):424-432.
199. Sunaga D, Tanno M, Kuno A, et al. Accelerated recovery of mitochondrial membrane potential by GSK-3 β inactivation affords cardiomyocytes protection from oxidant-induced necrosis. *PLoS One*. 2014;9(11):e112529.
200. Mancinelli R, Carpino G, Petrunaro S, et al. Multifaceted roles of GSK-3 in cancer and autophagy-related diseases. *Oxid Med Cell Longev*. 2017;2017:4629495.
201. Wang SH, Shih YL, Kuo TC, Ko WC, Shih CM. Cadmium toxicity toward autophagy through ROS-activated GSK-3 β in mesangial cells. *Toxicol Sci*. 2009;108(1):124-131.
202. Lin CJ, Chen TH, Yang LY, Shih CM. Resveratrol protects astrocytes against traumatic brain injury through inhibiting apoptotic and autophagic cell death. *Cell Death Dis*. 2014;5:e1147.
203. Liu L, Li CJ, Lu Y, et al. Baclofen mediates neuroprotection on hippocampal CA1 pyramidal cells through the regulation of autophagy under chronic cerebral hypoperfusion. *Sci Rep*. 2015;5:14474.
204. Ciotti S, Iuliano L, Cefalù S, et al. GSK3 β is a key regulator of the ROS-dependent necrotic death induced by the quinone DMNQ. *Cell Death Dis*. 2020;11(1):2.
205. Guidotti S, Minguzzi M, Platano D, et al. Glycogen synthase kinase-3 β inhibition links mitochondrial dysfunction, extracellular matrix remodelling and terminal differentiation in chondrocytes. *Sci Rep*. 2017;7(1):12059.
206. Guidotti S, Minguzzi M, Platano D, et al. Lithium chloride dependent glycogen synthase kinase 3 inactivation links oxidative DNA damage, hypertrophy and senescence in human articular chondrocytes and reproduces chondrocyte phenotype of obese osteoarthritis patients. *PLoS One*. 2015;10(11):e0143865.
207. Byun HO, Jung HJ, Seo YH, et al. GSK3 inactivation is involved in mitochondrial complex IV defect in transforming growth factor (TGF) β 1-induced senescence. *Exp Cell Res*. 2012;318(15):1808-1819.
208. Seo YH, Jung HJ, Shin HT, et al. Enhanced glycogenesis is involved in cellular senescence via GSK3/GS modulation. *Aging cell*. 2008;7(6):894-907.
209. Kim YM, Seo YH, Park CB, Yoon SH, Yoon G. Roles of GSK3 in metabolic shift toward abnormal anabolism in cell senescence. *Ann N Y Acad Sci*. 2010;1201:65-71.
210. Yoshida H. ER stress and diseases. *FEBS J*. 2007;274(3):630-658.
211. Almanza A, Carlesso A, Chintha C, et al. Endoplasmic reticulum stress signalling—from basic mechanisms to clinical applications. *FEBS J*. 2019;286(2):241-278.
212. Chen CH, Shaikhenov T, Peterson TR, et al. ER stress inhibits mTORC2 and Akt signaling through GSK-3 β -mediated phosphorylation of rictor. *Sci Signal*. 2011;4(161):ra10.
213. McAlpine CS, Bowes AJ, Khan MI, Shi Y, Werstuck GH. Endoplasmic reticulum stress and glycogen synthase kinase-3 β activation in apolipoprotein E-deficient mouse models of accelerated atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2012;32(1):82-91.
214. Koga T, Suico MA, Shimasaki S, et al. Endoplasmic reticulum (ER) stress induces sirtuin 1 (SIRT1) expression via the PI3K-Akt-GSK3 β signaling pathway and promotes hepatocellular injury. *J Biol Chem*. 2015;290(51): 30366-30374.
215. Meares GP, Mines MA, Beurel E, et al. Glycogen synthase kinase-3 regulates endoplasmic reticulum (ER) stress-induced CHOP expression in neuronal cells. *Exp Cell Res*. 2011;317(11):1621-1628.
216. Chen L, Ren F, Zhang H, et al. Inhibition of glycogen synthase kinase 3 β ameliorates D-GalN/LPS-induced liver injury by reducing endoplasmic reticulum stress-triggered apoptosis. *PLoS One*. 2012;7(9):e45202.
217. Kim S, Joe Y, Kim HJ, et al. Endoplasmic reticulum stress-induced IRE1 α activation mediates cross-talk of GSK-3 β and XBP-1 to regulate inflammatory cytokine production. *J Immunol*. 2015;194(9):4498-4506.

218. Hadj Ayed Tka K, Mahfoudh Boussaid A, Zaouali MA, et al. Melatonin modulates endoplasmic reticulum stress and Akt/GSK3-beta signaling pathway in a rat model of renal warm ischemia reperfusion. *Anal Cell Pathol (Amst)*. 2015;2015:635172.
219. Pluquet O, Qu LK, Baltzis D, Koromilas AE. Endoplasmic reticulum stress accelerates p53 degradation by the cooperative actions of Hdm2 and glycogen synthase kinase 3beta. *Mol Cell Biol*. 2005;25(21):9392-9405.
220. Nie T, Yang S, Ma H, et al. Regulation of ER stress-induced autophagy by GSK3beta-TIP60-ULK1 pathway. *Cell Death Dis*. 2016;7(12):e2563.
221. Martin M, Rehani K, Jope RS, Michalek SM. Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3. *Nat Immunol*. 2005;6(8):777-784.
222. Hu X, Paik PK, Chen J, et al. IFN-gamma suppresses IL-10 production and synergizes with TLR2 by regulating GSK3 and CREB/AP-1 proteins. *Immunity*. 2006;24(5):563-574.
223. Beurel E, Jope RS. Glycogen synthase kinase-3 promotes the synergistic action of interferon-gamma on lipopolysaccharide-induced IL-6 production in RAW264.7 cells. *Cell Signal*. 2009;21(6):978-985.
224. Beurel E, Jope RS. Differential regulation of STAT family members by glycogen synthase kinase-3. *J Biol Chem*. 2008;283(32):21934-21944.
225. Cheng Y, Pardo M, Armini RS, et al. Stress-induced neuroinflammation is mediated by GSK3-dependent TLR4 signaling that promotes susceptibility to depression-like behavior. *Brain Behav Immun*. 2016;53:207-222.
226. Zhao J, Wang H, Huang Y, et al. Lupus nephritis: glycogen synthase kinase 3beta promotion of renal damage through activation of the NLRP3 inflammasome in lupus-prone mice. *Arthritis Rheumatol*. 2015;67(4):1036-1044.
227. Liu W, Wang H, Wang Y, Li H, Ji L. Metabolic factors-triggered inflammatory response drives antidepressant effects of exercise in CUMS rats. *Psychiatry Res*. 2015;228(3):257-264.
228. Schroder K, Tschopp J. The inflammasomes. *Cell*. 2010;140(6):821-832.
229. Schroder K, Zhou R, Tschopp J. The NLRP3 inflammasome: a sensor for metabolic danger? *Science*. 2010;327(5963):296-300.
230. Jin C, Henao-Mejia J, Flavell RA. Innate immune receptors: key regulators of metabolic disease progression. *Cell Metab*. 2013;17(6):873-882.
231. Schwartz MW, Woods SC, Porte D Jr, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature*. 2000;404(6778):661-671.
232. Park SH, Ryu SY, Yu WJ, et al. Leptin promotes K(ATP) channel trafficking by AMPK signaling in pancreatic beta-cells. *Proc Natl Acad Sci USA*. 2013;110(31):12673-12678.
233. Park SH, Ho WK, Jeon JH. AMPK regulates K(ATP) channel trafficking via PTEN inhibition in leptin-treated pancreatic beta-cells. *Biochem Biophys Res Commun*. 2013;440(4):539-544.
234. Ning K, Miller LC, Laidlaw HA, et al. Leptin-dependent phosphorylation of PTEN mediates actin restructuring and activation of ATP-sensitive K⁺ channels. *J Biol Chem*. 2009;284(14):9331-9340.
235. Yang F, Wu R, Jiang Z, et al. Leptin increases mitochondrial OPA1 via GSK3-mediated OMA1 ubiquitination to enhance therapeutic effects of mesenchymal stem cell transplantation. *Cell Death Dis*. 2018;9(5):556.
236. Valerio A, Ghisi V, Dossena M, et al. Leptin increases axonal growth cone size in developing mouse cortical neurons by convergent signals inactivating glycogen synthase kinase-3beta. *J Biol Chem*. 2006;281(18):12950-12958.
237. Greco SJ, Sarkar S, Casadesus G, et al. Leptin inhibits glycogen synthase kinase-3beta to prevent tau phosphorylation in neuronal cells. *Neurosci Lett*. 2009;455(3):191-194.
238. Pérez C, Fernández-Galaz C, Fernández-Agulló T, et al. Leptin impairs insulin signaling in rat adipocytes. *Diabetes*. 2004;53(2):347-353.
239. Benzler J, Andrews ZB, Pracht C, et al. Hypothalamic WNT signalling is impaired during obesity and reinstated by leptin treatment in male mice. *Endocrinology*. 2013;154(12):4737-4745.
240. Jiang J, Fan Y, Zhang W, et al. Adiponectin suppresses human pancreatic cancer growth through attenuating the beta-catenin signaling pathway. *Int J Biol Sci*. 2019;15(2):253-264.
241. Wang Y, Lam JB, Lam KS, et al. Adiponectin modulates the glycogen synthase kinase-3beta/beta-catenin signaling pathway and attenuates mammary tumorigenesis of MDA-MB-231 cells in nude mice. *Cancer Res*. 2006;66(23):11462-11470.
242. Motawi TM, Bustanji Y, El-Maraghy SA, Taha MO, Al Ghoussein MA. Naproxen and cromolyn as new glycogen synthase kinase 3beta inhibitors for amelioration of diabetes and obesity: an investigation by docking simulation and subsequent in vitro/in vivo biochemical evaluation. *J Biochem Mol Toxicol*. 2013;27(9):425-436.
243. Chen H, Fajol A, Hoene M, et al. PI3K-resistant GSK3 controls adiponectin formation and protects from metabolic syndrome. *Proc Natl Acad Sci USA*. 2016;113(20):5754-5759.
244. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. *Nature*. 2005;434(7029):113-118.

245. Lagouge M, Argmann C, Gerhart-Hines Z, et al. Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1 α . *Cell*. 2006;127(6):1109-1122.
246. Ramadori G, Fujikawa T, Fukuda M, et al. SIRT1 deacetylase in POMC neurons is required for homeostatic defenses against diet-induced obesity. *Cell Metab*. 2010;12(1):78-87.
247. Li XH, Chen C, Tu Y, et al. Sirt1 promotes axonogenesis by deacetylation of Akt and inactivation of GSK3. *Mol Neurobiol*. 2013;48(3):490-499.
248. Dan L, Klimenkova O, Klimiankou M, et al. The role of sirtuin 2 activation by nicotinamide phosphoribosyltransferase in the aberrant proliferation and survival of myeloid leukemia cells. *Haematologica*. 2012;97(4):551-559.
249. Si X, Chen W, Guo X, et al. Activation of GSK3 β by Sirt2 is required for early lineage commitment of mouse embryonic stem cell. *PLoS One*. 2013;8(10):e76699.
250. Sarikhani M, Mishra S, Maity S, et al. SIRT2 deacetylase regulates the activity of GSK3 isoforms independent of inhibitory phosphorylation. *eLife*. 2018;7:e32952.
251. Monteserin-Garcia J, Al-Massadi O, Seoane LM, et al. Sirt1 inhibits the transcription factor CREB to regulate pituitary growth hormone synthesis. *FASEB J*. 2013;27(4):1561-1571.
252. Vachharajani VT, Liu T, Wang X, Hoth JJ, Yoza BK, McCall CE. Sirtuins Link Inflammation and Metabolism. *J Immunol Res*. 2016;2016:8167273.
253. Lin SC, Hardie DG. AMPK: Sensing Glucose as well as Cellular Energy Status. *Cell Metab*. 2018;27(2):299-313.
254. Garcia D, Shaw RJ. AMPK: mechanisms of cellular energy sensing and restoration of metabolic balance. *Mol Cell*. 2017;66(6):789-800.
255. Suzuki T, Bridges D, Nakada D, et al. Inhibition of AMPK catabolic action by GSK3. *Mol Cell*. 2013;50(3):407-419.
256. Park DW, Jiang S, Liu Y, et al. GSK3 β -dependent inhibition of AMPK potentiates activation of neutrophils and macrophages and enhances severity of acute lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2014;307(10):L735-L745.
257. Park SY, Lee YK, Lee WS, Park OJ, Kim YM. The involvement of AMPK/GSK3- β signals in the control of metastasis and proliferation in hepato-carcinoma cells treated with anthocyanins extracted from Korea wild berry Meoru. *BMC Complement Altern Med*. 2014;14:109.
258. Horike N, Sakoda H, Kushiyama A, et al. AMP-activated protein kinase activation increases phosphorylation of glycogen synthase kinase 3 β and thereby reduces cAMP-responsive element transcriptional activity and phosphoenolpyruvate carboxykinase C gene expression in the liver. *J Biol Chem*. 2008;283(49):33902-33910.
259. Delcommenne M, Tan C, Gray V, Rue L, Woodgett J, Dedhar S. Phosphoinositide-3-OH kinase-dependent regulation of glycogen synthase kinase 3 and protein kinase B/AKT by the integrin-linked kinase. *Proc Natl Acad Sci USA*. 1998;95(19):11211-11216.
260. Miller JR, Moon RT. Signal transduction through beta-catenin and specification of cell fate during embryogenesis. *Genes Dev*. 1996;10(20):2527-2539.
261. Fang X, Yu SX, Lu Y, Bast RC Jr, Woodgett JR, Mills GB. Phosphorylation and inactivation of glycogen synthase kinase 3 by protein kinase A. *Proc Natl Acad Sci USA*. 2000;97(22):11960-11965.
262. Zhang HH, Lipovsky AI, Dibble CC, Sahin M, Manning BD. S6K1 regulates GSK3 under conditions of mTOR-dependent feedback inhibition of Akt. *Mol Cell*. 2006;24(2):185-197.
263. Armstrong JL, Bonavaud SM, Toole BJ, Yeaman SJ. Regulation of glycogen synthesis by amino acids in cultured human muscle cells. *J Biol Chem*. 2001;276(2):952-956.
264. Um SH, Frigerio F, Watanabe M, et al. Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature*. 2004;431(7005):200-205.
265. Tremblay F, Krebs M, Dombrowski L, et al. Overactivation of S6 kinase 1 as a cause of human insulin resistance during increased amino acid availability. *Diabetes*. 2005;54(9):2674-2684.
266. He L, Gomes AP, Wang X, et al. mTORC1 promotes metabolic reprogramming by the suppression of GSK3-dependent Foxk1 phosphorylation. *Mol Cell*. 2018;70(5):949-960.
267. Ka M, Condorelli G, Woodgett JR, Kim WY. mTOR regulates brain morphogenesis by mediating GSK3 signaling. *Development*. 2014;141(21):4076-4086.
268. Inoki K, Ouyang H, Zhu T, et al. TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. *Cell*. 2006;126(5):955-968.
269. Stretton C, Hoffmann TM, Munson MJ, et al. GSK3-mediated raptor phosphorylation supports amino-acid-dependent mTORC1-directed signalling. *Biochem J*. 2015;470(2):207-221.
270. Ramirez-Valle F, Badura ML, Braunstein S, Narasimhan M, Schneider RJ. Mitotic raptor promotes mTORC1 activity, G(2)/M cell cycle progression, and internal ribosome entry site-mediated mRNA translation. *Mol Cell Biol*. 2010;30(13):3151-3164.
271. Sayas CL, Moreno-Flores MT, Avila J, Wandosell F. The neurite retraction induced by lysophosphatidic acid increases Alzheimer's disease-like Tau phosphorylation. *J Biol Chem*. 1999;274(52):37046-37052.

272. Ding VW, Chen RH, McCormick F. Differential regulation of glycogen synthase kinase 3beta by insulin and Wnt signaling. *J Biol Chem*. 2000;275(42):32475-32481.
273. Kimelman D, Xu W. beta-catenin destruction complex: insights and questions from a structural perspective. *Oncogene*. 2006;25(57):7482-7491.
274. Lee E, Salic A, Krüger R, Heinrich R, Kirschner MW. The roles of APC and Axin derived from experimental and theoretical analysis of the Wnt pathway. *PLoS Biol*. 2003;1(1):E10.
275. Salic A, Lee E, Mayer L, Kirschner MW. Control of beta-catenin stability: reconstitution of the cytoplasmic steps of the wnt pathway in *Xenopus* egg extracts. *Mol Cell*. 2000;5(3):523-532.
276. Liu J, Stevens J, Rote CA, et al. Siah-1 mediates a novel beta-catenin degradation pathway linking p53 to the adenomatous polyposis coli protein. *Mol Cell*. 2001;7(5):927-936.
277. Topol L, Jiang X, Choi H, Garrett-Beal L, Carolan PJ, Yang Y. Wnt-5a inhibits the canonical Wnt pathway by promoting GSK-3-independent beta-catenin degradation. *J Cell Biol*. 2003;162(5):899-908.
278. Tolwinski NS, Wehrli M, Rives A, Erdeniz N, DiNardo S, Wieschaus E. Wg/Wnt signal can be transmitted through arrow/LRP5,6 and Axin independently of Zw3/Gsk3beta activity. *Dev Cell*. 2003;4(3):407-418.
279. Anmella G, Fico G, Lotfaliany M, et al. Risk of cancer in bipolar disorder and the potential role of lithium: international collaborative systematic review and meta-analyses. *Neurosci Biobehav Rev*. 2021;126:529-541.
280. Beaulieu JM, Marion S, Rodriguiz RM, et al. A beta-arrestin 2 signaling complex mediates lithium action on behavior. *Cell*. 2008;132(1):125-136.

How to cite this article: Wang L, Li J, Di L. Glycogen synthesis and beyond, a comprehensive review of GSK3 as a key regulator of metabolic pathways and a therapeutic target for treating metabolic diseases. *Med Res Rev*. 2022;42:946-982. doi:10.1002/med.21867