GeroScience (2019) 41:369–382
https://doi.org/10.1007/s11357-019-00085-z

REVIEW ARTICLE

An expanding GSK3 network: implications for aging research

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Received: 28 May 2019 / Accepted: 2 July 2019 / Published online: 17 July 2019

Abstract The last few decades of longevity research have been very exciting. We now know that longevity and healthspan can be manipulated across species, from unicellular eukaryotes to nonhuman primates, and that while aging itself is inevitable, how we age is malleable. Numerous dietary, genetic, and pharmacological studies now point to links between metabolism and growth regulation as a central aspect in determining longevity and, perhaps more importantly, health with advancing age. Here, we focus on a relatively new player in aging studies GSK3, glycogen synthase kinase, a key factor in growth and metabolism whose name fails to convey the extensive breadth of its role in cellular adaptation. First, we provide a brief overview of GSK3, touching on those aspects that are likely relevant to aging. Then, we outline the role of GSK3 in cellular functions including growth signaling, cell fate, and metabolism. Next, we describe evidence demonstrating a direct role for GSK3 in a range of age-related diseases, despite the fact that they differ considerably in their etiology and pathology. Finally, we discuss the role that GSK3 may play in normative aging and how GSK3 might be a suitable target to oppose age-related disease vulnerability.

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Keywords GSK3 · Glycogen synthase kinase 3 · Aging · Metabolism · Age-related disease

Introduction

Aging is the greatest risk factor for a range of chronic diseases and disorders including cancer, diabetes, and neurodegenerative disease, and significant effort is being invested to identify casual aspects in morbidity and loss of resilience as a function of age (Kennedy et al. 2014). Dietary excess and a sedentary lifestyle appear to increase vulnerability to diseases and disorders traditionally viewed as age-related (Bonomini et al. 2015), potentially linking the pace of aging to metabolic dysfunction. Caloric restriction (CR) without malnutrition prolongs lifespan and healthspan in a wide range of species including non-human primates, and although the mechanisms yet to be established, a growing literature points to a central role for metabolism in the beneficial effects of CR (Balasubramanian et al. 2017). A prevailing theme in genetic studies of aging is that repression of growth and growth signaling is also strongly linked to longevity (Bartke 2017). Evidence from yeast, worms, flies, and rodents links lifespan extension to insulin and IGF-1 signaling pathway components (Fontana and Partridge 2015). Pathways regulating growth signaling and metabolism are known to be highly interconnected, raising the possibility that this integrated network is what is intrinsically linked to the increase in disease vulnerability that accompanies age. Factors that coordinate growth signaling and

metabolism are strong candidates as targets in the treatment of age-related diseases and in development of preventative interventions to prolong good health with advancing age. One such effector of growth signaling is glycogen synthase-kinase 3 (GSK3), a broad specificity serine-threonine kinase that has been linked to insulin resistance, systemic inflammation, and several aspects of Alzheimer's disease (AD) pathology (Beurel et al. 2015).

There are two isoforms of GSK3 enzyme, GSK3a and GSK3b, collectively referred to as GSK3. The genes encoding these two isoforms reside on separate chromosomes and are ubiquitously expressed (Woodgett 1990). Loss of function mutants have revealed that they have partially overlapping functions; GSK3a knockout (KO) mice are viable due to compensatory activity from GSK3b, but GSK3b KO is embryonically lethal (Hoeflich et al. 2000; Kaidanovich-Beilin et al. 2009; MacAulay et al. 2007). Genetic studies have place GSK3 as a critical regulator of growth and development that also impinges on metabolic homeostatic mechanisms (Table 1). GSK3 is constitutively active and can be inhibited through phosphorylation or by sequestration in a cytosolic complex (Cross et al. 1995; Dominguez et al. 1995). Signaling through insulin and WNT pathways appears to regulate distinct pools of GSK3: AKT activation leads to GSK3 phosphorylation and inhibition but does not affect beta-catenin, while WNT causes dislocation of GSK3 from its beta-catenin targeting destruction complex, leading to stabilized and active beta-catenin (Ding et al. 2000). Despite these differences in mechanistic detail, both WNT and insulin pathways share GSK3b as an effector in signaling and both converge on cell growth and metabolism. Over 70 GSK3 substrates have been validated, representing diverse roles in cellular function. Many GSK3b targets have established relevance to aging, including PI3K, mTOR complex 1 (mTORC1), AMP kinase (AMPK), and peroxisome proliferator-activated receptor gamma co-activator 1alpha (PGC-1a), among others. This breadth of influence implies that GSK3 may be a central coordinator of the cellular response to growth stimulus or repression (Sutherland 2011). Genetic studies have revealed general details of GSK3 function at the cellular level, including signaling downstream of growth and inflammation, and modulation of cell cycle (Jope and Johnson 2004; Jope et al. 2017). GSK3b in particular is enriched in the brain where it has brain-specific roles and is required for neurogenesis, regulation of synaptic plasticity, and neurotransmission (Beurel et al. 2015). These aspects of GSK3b have been particularly well studied in the context of psychiatric disorders, where the GSK3b inhibitor lithium has been used for over a century as a mood-stabilizing agent (Klein and Melton 1996). Intriguingly, GSK3b has also been identified as a major tau kinase implicated in the formation of neurofibrillary tangles, making it a potential target for AD therapeutics (Hanger et al. 1992). As outlined below, recent evidence implicates GSK3b in models of delayed and accelerated aging and interesting new roles for GSK3b in cellular function have been discovered. The goal of this review is to shed light on GSK3b as a factor that links metabolism and growth signaling, and to discuss how it might play a role in aging as a driver of age-related pathology.

GSK3 and growth signaling

Upstream mechanisms controlling GSK3 activity, including protein complex formation, sequestration, and autoregulation, have been thoroughly reviewed elsewhere (Beurel et al. 2015). Briefly, inhibitory phosphorylation of GSK3b occurs at the serine-9 position in response to growth stimulus, subsequently impacting GSK3 target proteins involved in glycogen synthesis, translation, and cell survival (Stambolic and Woodgett 1994; Frame and Cohen 2001). Many GSK3 substrates require priming phosphorylation four residues C-terminal to the GSK3 consensus site (S/TXXXS/T) and serine-9 phosphorylation of GSK3 only prevents the binding of the kinase to primed substrates (Frame and Cohen 2001). Both AKT and S6K have been shown to phosphorylate GSK3b at the serine 9 site (serine X in SK3a) in response to growth signaling, while phosphatases including PP2A result in dephosphorylation at this site (Stambolic and Woodgett 1994; Cross et al. 1995; Eldar-Finkelman et al. 1995). Interestingly PI3K and mTOR complex 1 are key nodes that regulate longevity, and significant cross-talk between these pathways has been heavily implicated in upstream regulation of GSK3 activity (Manning and Toker 2017). More recent evidence also places both mTOR complex 1 and PI3K signaling downstream of GSK3, revealing a complex network of signals that impacts cellular function and health (Hermida et al. 2017).

GSK3 phosphorylates and activates TSC2 resulting in inhibition of the GTPase Rheb with subsequent inhibition of the mTOR complex 1 pathway. Interestingly,

Table 1 Transgenic mouse models reveal the biology of GSK3

PMID	Year	Isoform	Expression system	Functional outcomes
GSK3 gain of function				
11007782	2000	GSK3b	Thy1-Cre	Tau hyperphosphorylation
12182887	2002	(S9A)		Reduced brain size
16943560	2006			Hypophagia, increased locomotor activity
11226152	2001	GSK3b	CamkIIa-Cre (Tet-off)	Astrogliosis, apoptosis, and neurodegeneration
12472906	2002			Impaired spatial memory
17241269	2007			Impaired long-term potentiation
15375789	2004	GSK3b	a-actin-Cre	Impaired glucoregulation, hyperlipidemia, and adiposity
15791206	2005	GSK3a (S21A)	Whole body	Normal glucoregulation
27140617	2016	GSK3b (S9A)		Resistance to HFD-induced adiposity and glucoregulatory dysfunction, increased adiponectin
18219478	2008	GSK3b (S9A)	Insulin2-Cre	Reduced beta-cell mass, impaired glucoregulation
GSK3 loss of function				
10894547	2000	GSK3b	Whole body	Embryonic lethality mediated by TNFa
17908561	2007	GSK3a	Whole body	Enhanced glucoregulation and IRS1 expression
19925672	2009			Reduced aggression, impaired motor coordination
23549082	2013			Reduced lifespan, sarcopenia, increased cellular senescence
18694957	2008	GSK3b	Albumin-Cre	Normal glucoregulation
			MLC1*f*-Cre	Enhanced glucoregulation and skeletal muscle glycogen
19801986	2009	GSK3a GSK3b	Whole body Nestin-Cre	Progenitor pool expansion, impaired neurogenesis
20821187	2010	GSK3b	Insulin2-Cre	Enhanced glucoregulation, resistance to HFD-induced diabetes

expression of TSC2 lacking the GSK3b phosphorylation sites resulted in persistent activation of mTOR complex 1 and apoptosis in response to nutrient deprivation (Inoki et al. 2006). This potentially places GSK3b upstream of other mTORC1 functions, including lipid and protein biosynthesis, nucleotide metabolism, and glycolysis; however, the role of GSK3 in controlling cell activity at the functional level has not been very well defined. Glucose uptake and coincident GLUT1 expression is increased in response to GSK3 inhibition in vascular smooth muscle (Buller et al. 2008). Translation is reportedly blocked by GSK3b activity, but it is unclear whether this effect is mediated by mTOR complex 1 inhibition or by direct phosphorylation of eIF2B, another GSK3b substrate (Welsh et al. 1998). More recently, GSK3 has been implicated as a negative regulator of mTOR complex 2 function, where the complex 2 binding partner Rictor was identified as a GSK3 substrate that is subsequently targeted for proteasomal degradation (Koo et al. 2015). GSK3 itself has also been implicated as a target downstream of mTOR complex 1. TSC2 deficiency in cells results in persistent S6K-dependent inhibition of GSK3b, although this regulatory mechanism may only be relevant in the context of insulin resistance, since in these cells signaling through insulin pathways was blunted and AKT, the usual dominant kinase for GSK3, was inhibited (Zhang et al. 2006). Altogether, these complex interactions with insulin and mTOR demonstrate that GSK3b is important in redirecting cellular growth and synthetic pathways in response to changes in nutrient availability (Fig.1).

In addition to controlling aspects of mTOR signaling, GSK3 has multiple interactions within the insulin/IGF1 signaling pathway. Although GSK3 is canonically inhibited through insulin signaling, it also exerts feedback control on this pathway. GSK3 was shown to phosphorylate IRS-1 and suppress insulin signaling activity in cells (Liberman and Eldar-Finkelman 2005). Inhibitory phosphorylation by GSK3 has also been extended to IRS-2 in the stress response (Sharfi and Eldar-Finkelman 2008). In another study, GSK3 was found to target IRS-1 for proteasomal degradation under conditions of insulin resistance, demonstrating that GSK3

controls both the stability and activity of this protein (Leng et al. 2010) (Fig.2). The stability of the insulin-signaling antagonist PTEN is also regulated by GSK3 phosphorylation, although the functional consequences of this interaction are unclear (Maccario et al. 2007). These data collectively implicate GSK3 as a regulator of insulin sensitivity that may be mechanistically important in conditions such as type 2 diabetes. This notion is supported by data showing that GSK3 can impact key aspects of glucoregulatory health and is discussed in more detail below.

GSK3 and cell fate

Downstream of its involvement in growth and nutrient signaling, GSK3b directly impacts cell survival and proliferation (Fig.3). Early work demonstrated that GSK3b activation was necessary for growth factor withdrawal-induced apoptosis, and has since been extended to a wide variety of toxicities, including oxidative stress, ER stress, and neuronal excitotoxicity (Maurer et al. 2014). MCL-1 (myeloid leukemia cell differentiation protein) is targeted for degradation by GSK3b in response to both inflammatory stimuli and growth factor withdrawal, resulting in activation of the intrinsic apoptotic pathway (Maurer et al. 2006). GSK3 additionally phosphorylates VDAC1, which blocks its interaction with hexokinase II. The loss of interaction disrupts mitochondrial localization and anti-apoptotic function of hexokinase II and increases the vulnerability of cells to apoptosis (Pastorino et al. 2005). It was subsequently shown that GSK3 is a positive regulator of the mitochondrial permeability transition pore (mPTP). GSK3b association with VDAC2 is necessary for maximal permeability of the mPTP under conditions of oxidative stress (Tanno et al. 2014). Intriguingly, GSK3b also upregulates activity of the tumor suppressor TP53, resulting in cell cycle arrest and apoptosis (Watcharasit et al. 2003). In contrast to the pro-apoptotic effects of GSK3b activity, GSK3b inhibition through WNT and growth signaling promotes cell survival and proliferation. The effect of GSK3b on cell proliferation has been demonstrated in stem cells, where inhibition of GSK3b promotes self-renewal and blocks differentiation (Kim et al. 2009). Deletion of both GSK3a and GSK3b in neural progenitor cells resulted in a massive expansion of progenitor cells; however, these progenitors showed compromised differentiation. GSK3-directed deregulation of stem cells was linked to beta-catenin, although activation of c-myc and c-jun that are also direct substrates of GSK3 could reasonably play a role too (Sutherland 2011). Expression of constitutively active GSK3 mutants resistant to N-terminal phosphorylation (mutated at S21A and S9A for GSK3a and GSK3b respectively) results in blockage of neural precursor cell proliferation (Eom and Jope 2009). The ability of GSK3 control cell fate has significant implications for the aging field: stem cell and progenitor cell renewal declines with age resulting in a diminished capacity of tissues to regenerate (Lopez-Otin et al. 2013). Taken together, these multiple interactions ensure that GSK3b can rapidly induce changes in cell survival in response to extracellular stimuli or when conditions are appropriate can stimulate renewal through stem cell recruitment. Therapeutic inhibition of GSK3 has the potential to counteract age-related decline in cellular renewal and stem cell recruitment; however, given the role of GSK3 in the balance of growth and cell survival, the risks of such a therapy would need to be thoroughly evaluated.

GSK3 and inflammation

Chronic inflammation increases with age and growth signaling pathways intersect with inflammation through GSK3. The regulation of inflammation by GSK3 is best characterized through its differential control of CREB and NF-kB transcriptional activity (Fig.4). GSK3 is required for the integrity of Toll-like receptor (TLR) signaling (Martin et al. 2005). Downstream of TLR activation, the status of GSK3 tips the balance between pro- and anti-inflammatory cytokine production. GSK3 activity is required for production of pro-inflammatory IL-6, IL-1B, and IFNy following TLR stimulation, and inhibition of GSK3 during TLR activation favors IL-10 production. Mechanistically, these outcomes are linked to increased nuclear CREB activity and lower NFkB activity in GSK3-inhibited TLR-activated monocytes, where CREB competes and sequesters CPB, a binding partner it shares with NFkB. Inhibition of GSK3 with lithium has also been shown to elevate CREB activity in neuronal cells (Grimes and Jope 2001), supporting the notion that GSK3 could regulate neuroinflammation. This is supported by in vivo evidence showing that adult onset neuron-specific overexpression of GSK3b (CamKII Tet) induces astrogliosis; however, this effect appears to be cell non-autonomous as glia do not express the transgene (Lucas et al. 2001)(Table 1). Both

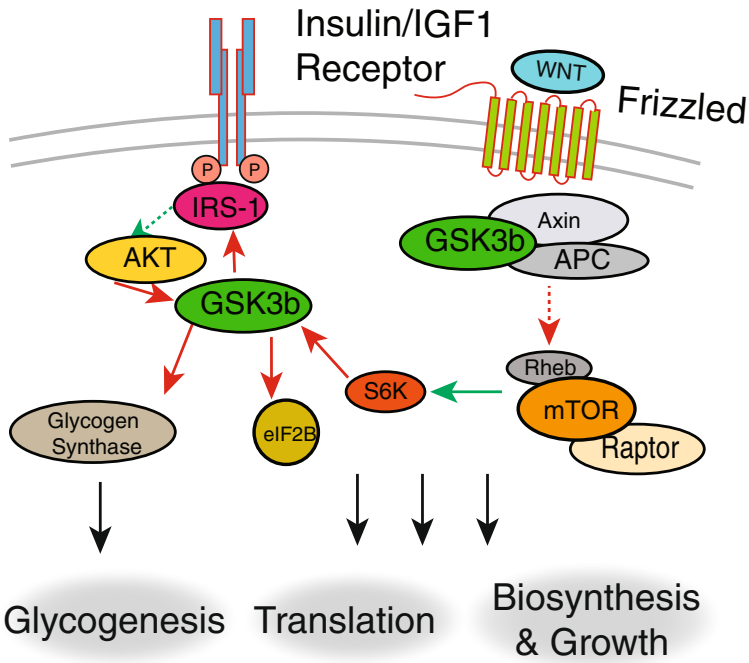

Fig. 1 GSK3b and growth regulation. GSK3b activity is suppressed by the insulin/IGF1 and WNT signaling pathways. In the resting state, GSK3b is constitutively active and suppresses growth signaling, translation, and glycogenesis by phosphorylating multiple targets. Activation of Akt by the insulin signaling pathway leads to phosphorylation and suppression of GSK3b activity. GSK3b is also active in the absence of WNT signaling leading to inhibition of mTORC1 and cell growth. Conversely, active mTORC1 inhibits GSK3b activity in a negative feedback loop through S6K

CREB and NFkB have been identified as direct substrates of GSK3b. Phosphorylation of CREB occurs after priming phosphorylation by PKA, preventing CREB DNA binding and activity thus ensuring that CREB activity is temporally controlled (Bullock and Habener 1998; Grimes and Jope 2001). NEMO is an indirect NFkB regulator that along with IKK targets NFkB inhibitor IkB for proteasomal degradation. NEMO interacts with and is phosphorylated by GSK3b at multiple sites resulting in its stabilization (Medunjanin et al. 2016). Importantly, neuroinflammation has been linked to peripheral metabolic dysfunction in mice (Zhang et al. 2008), and it is not unreasonable to think it could also contribute to pathological aging

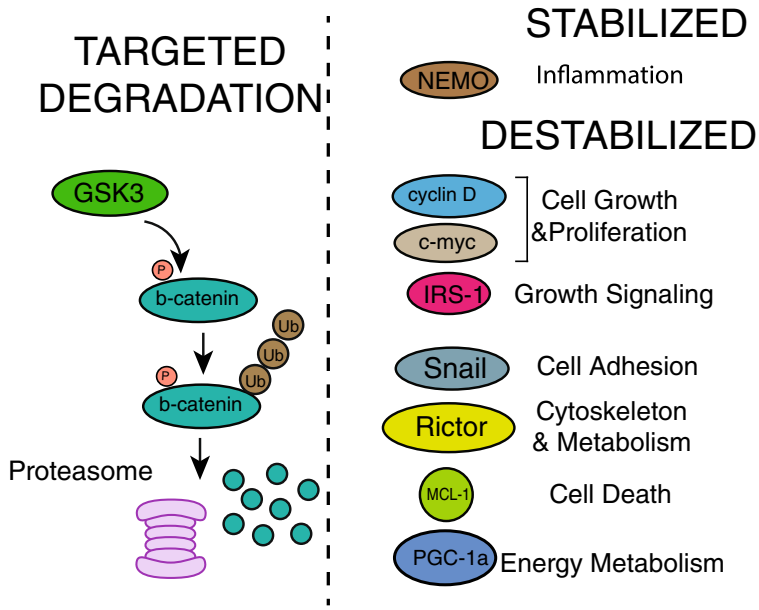

Fig. 2 GSK3 phosphorylation regulates the stability of diverse protein targets. Many proteins phosphorylated by GSK3b are targeted for ubiquitin-mediated degradation, most notably beta-catenin in the absence of WNT signaling. These proteins are involved in diverse pathways that impact aging and disease vulnerability including inflammation, metabolism, growth, and different aspects of cell fate

through this same IKK/NFkB axis. Cross-talk between growth and inflammation has been established in Th17 cells through GSK3a. Here, IKK is activated in response to IL-1 signaling resulting in GSK3a inhibition and stimulation of AKT-mTORC1 (Gulen et al. 2012), linking GSK3 to regulation of adaptive immunity. The importance of GSK3 in mediating the inflammatory response is underscored by the effectiveness of GSK3 inhibitors in treating inflammation. Administration of a GSK3 inhibitor prevented the death of mice resulting from a lethal dose (LD_{100}) of lipopolysaccharide (Martin et al. 2005). Elsewhere, GSK3b plays a pro-inflammatory role in mouse models of arthritis and peritonitis (Hu et al. 2006). Here too GSK3 resides at the intersection of pro-and anti-inflammatory cytokine production and mediates cross-talk between Interferon gamma (IFNg) and TLR signaling. These studies suggest that GSK3 inhibitors could be therapeutically useful in the treatment of inflammatory diseases. In mouse models of accelerated aging, the observed increased inflammation and is associated with activation of NEMO (Osorio et al. 2012). Indeed, aging is associated with low-grade sterile inflammation (Franceschi and Campisi 2014) that includes elevated pro-inflammatory cytokines in the serum, activation of NF-kB signaling, and increased expression of immune and inflammation-related genes (Salminen et al. 2012). Aging studies investigating the impact of long-term GSK3 inhibition are needed to clarify whether targeting GSK3 could be useful in attenuating age-related increases in inflammation.

GSK3 and cellular senescence

Another age-related phenomenon linked to GSK3 is cellular senescence, a state of permanent cell cycle arrest caused by diverse adverse signals, including contact inhibition, genotoxic injury, and mitochondrial stress (Campisi and d'Adda di Fagagna 2007; Wiley et al. 2016). Transplantation of senescent cells into mice resulted in aging pathology, while removal of these cells pharmacologically alleviated symptoms suggesting that cell senescence directly contributes to the aging process (Xu et al. 2018). N-terminal phosphorylation of both GSK3 isoforms is reported to accompany hepatocyte senescence and treatment with lithium was sufficient to induce a senescent phenotype that is coincident with augmented anabolism including protein synthesis and glycogenesis (Seo et al. 2008). GSK3b was shown to accumulate in the nucleus of human fibroblasts that had undergone replicative senescence, where it formed a stable complex with p53 (Zmijewski and Jope 2004). Intriguingly, treatment of these cells with lithium blocked the interaction of GSK3b with p53 and caused cells to enter a reversible quiescent state. Thus, it appears GSK3 could both promote and oppose cellular senescence under different circumstances. The role of mitochondrial metabolism in cellular senescence has yet to be fully resolved. Mitochondrial dysfunction has been associated with senescence and is detected in senescent cells; however, mitochondrial dysfunction has also been identified as a causal agent in senescence (Kwon et al. 2019). Growing evidence shows a role for cellular senescence and the senescence-associated secretory phenotype in promoting aging phenotypes (Kirkland and Tchkonia 2017). GSK3, residing as it does at the intersection of growth and metabolism, is likely to be important in senescence and may even contribute senescent cell accumulation as a function of age.

GSK3 and energy metabolism

Growth signaling was one of the first pathways to be associated with longevity and the impact of growth inhibition been consistently observed among species (Fontana and Partridge 2015). Extended lifespan has been shown in several genetic mouse models of somatotropic axis deficiency (Bartke 2017), and blockage of growth by CR is thought to partially mediate its benefits (Anderson and Weindruch 2010). The reduction of growth with CR is accompanied by a coordinated upregulation of mitochondrial energy metabolism pathways. Intrigingly, this effect is highly conserved in multiple tissues in mice and among species on CR, suggesting that it is fundamentally important how CR works (Barger et al. 2015). An important question is how cross-talk between growth and metabolic pathways might mediate the effects of CR. As a key effector of growth signaling, GSK3 is perfectly positioned as a regulator of this cross-talk and recent studies have explored the role of both GSK3 isoforms in controlling key metabolic proteins, intermediary metabolism, and mitochondrial function.

AMPK is a major player in regulating cellular energetics (Steinberg and Carling 2019). GSK3b phosphorylation inhibits AMPK activity by making the activation loop site more vulnerable to inhibitory phosphatases (Suzuki et al. 2013). Ablation of the GSK3b site on

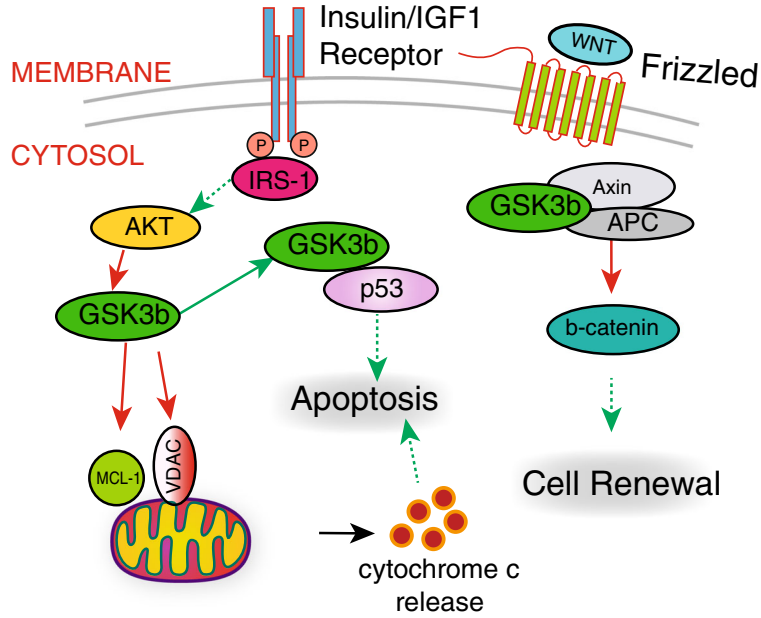

Fig. 3 GSK3 regulates cell death, renewal, and differentiation. Active GSK3b increases mitochondrial permeability by phosphorylating proteins on the mitochondrial outer membrane. Subsequent release of cytochrome c triggers the intrinsic pathway leading to apoptosis. GSK3b also interacts with p53 and promotes cell cycle arrest and apoptosis. Inhibitory phosphorylation of GSK3b in response to insulin signaling promotes cell survival by blocking these pathways. Stem cell function is regulated in part through the activity of the WNT signaling pathway. GSK3b phosphorylates and targets beta-catenin for degradation in the absence of WNT signaling, blocking stem cell renewal

AMPK in cells resulted in constitutive autophagy and inability to respond to anabolic conditions. Interestingly, GSK3b phosphorylation of AMPK requires a priming phosphorylation by AKT indicating that GSK3b may play a role in fine-tuning the balance of anabolism and catabolism to energetic status. Control over AMPK raises the possibility that GSK3 could be a negative regulator of mitochondrial energy production. GSK3 has previously been shown to inhibit the activity of pyruvate dehydrogenase, which may attenuate mitochondrial activity (Hoshi et al. 1996). A more direct relationship between GSK3 and mitochondria has been established through Drp1, which promotes mitochondrial fission (Chou et al. 2012). Phosphorylation of Drp1 by GSK3 promoted elongated mitochondrial morphology, while inhibition with lithium resulted in more fragmented mitochondria. The ability of GSK3 to regulate mitochondria through PGC-1a, a transcriptional coactivator and master regulator of mitochondrial function, has also been reported (Anderson et al. 2008), where GSK3b was shown to target PGC-1a for nuclear proteasomal degradation (Fig.5). Another study in primary neurons demonstrated that GSK3b phosphorylation of PGC-1a at T-295 was required for recognition by an E3 ubiquitin ligase (Olson et al. 2008). Several additional questions surrounding this regulation remain, including the identity of the kinase that primes PGC-1a for GSK3b targeting. In liver, GSK3b phosphorylates PPARa at serine 73, resulting in ubiquitination and proteasomal degradation. GSK3 inhibition in this study was associated with attenuated hepatic steatosis in high-fat-diet-fed mice, highlighting an important connection between GSK3b and metabolic disease (Hinds et al. 2016). The functional consequences of GSK3b manipulation have recently been investigated in the context of the brain. GSK3b is enriched in neurons, which are highly dependent on mitochondrial function. Brain aging is associated with changes in neuronal energy metabolism (Martin et al. 2016), and treatment of mice with GSK3 inhibitor lithium carbonate resulted in upregulation of PGC-1a and elevation of cytochrome c oxidase activity in the hippocampus (Martin et al. 2018). Inhibition of GSK3 stabilized PGC-1a protein and enhanced mitochondrial activity in neuroglioma cells, with concomitant changes in redox metabolism. Collectively, these studies support the idea that active GSK3 could regulate energy metabolism through multiple mechanisms. Identifying metabolic pathways downstream of

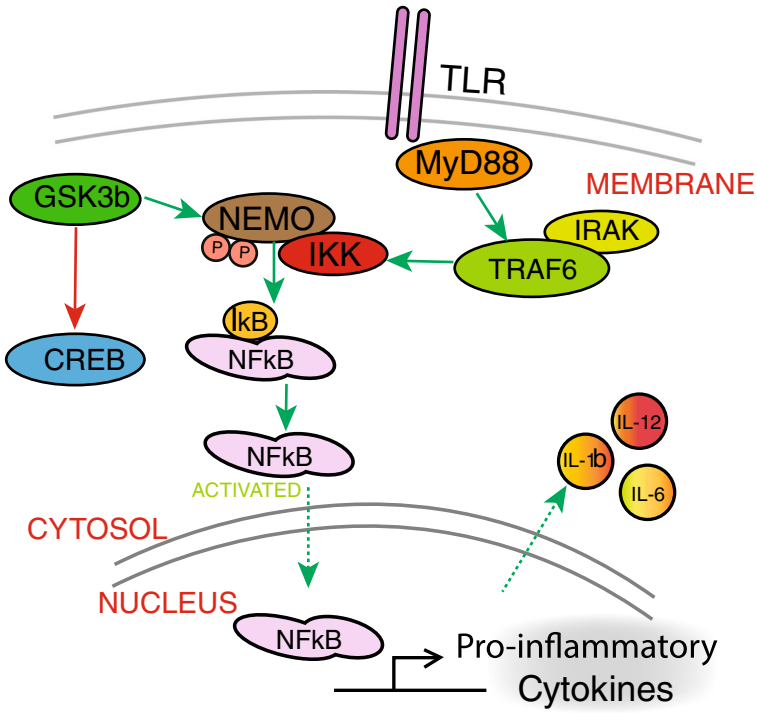

Fig. 4 GSK3b regulates inflammation. Active GSK3b promotes NFkB stability and nuclear localization downstream of toll-like receptor (TLR) signaling, leading to the production of pro-inflammatory cytokines. Conversely, GSK3b blocks nuclear accumulation of CREB by promoting its degradation. GSK3b also promotes the integrity of TLR signaling by stabilizing NEMO, a factor that promotes the DNA binding activity of NFkB

both GSK3 isoforms will open the door to therapeutics that target age-related metabolic impairment.

GSK3 and age-related disease

Changes in GSK3 expression and function are strongly related to several diseases and disorders that share an increase in risk of incidence as a function of age. Among these are type 2 diabetes, cancer, inflammatory conditions, and Alzheimer's disease (Beurel et al. 2015). There is a substantial volume of research published on each of these chronic conditions and the role of GSK3 in each is rather scattered about the literature. Here we will briefly describe some of the studies that favor a role for GSK3 in progression of age-related diseases and suggest that GSK3 could be an agent in creating age-related disease vulnerability in the first place.

Diabetes GSK3b expression levels have been found to correlate positively with glucoregulatory dysfunction in diabetes patients (Nikoulina et al. 2000). The significance of this association is unclear; however, genetic studies have revealed that GSK3 is a powerful mediator of systemic glucose and lipid homeostasis and that these actions have a high degree of tissue specificity (Table 1). The whole-body GSK3b KO is embryonic lethal but the whole-body GSK3a KO mice display improved glucoregulatory function early in life (MacAulay et al. 2007). Skeletal muscle-specific GSK3b overexpression resulted in hyperlipidemia, elevated fasting insulin, and glucose intolerance in male mice (Pearce et al. 2004). Conversely, mice lacking GSK3b in skeletal muscle exhibit improved glucose tolerance and greater insulin-stimulated glycogen synthase regulation (Patel et al. 2008). Liver-specific deletion of GSK3b did not exhibit an overt phenotype, indicating that the glucoregulatory role of GSK3 appears to be primarily extra-hepatic (Patel et al. 2008). It seems likely that at least some of the phenotypes GSK3 overexpression are linked to its role downstream of insulin and mTOR signaling. Double knock-in mice expressing AKT insensitive GSK3a and GSK3b were resistant to high-fat-diet-induced obesity, dyslipidemia, and glucoregulatory impairment (Chen et al. 2016). These mice also showed greater circulating levels of adiponectin, the adipose tissue-derived endocrine factor that signals through AMPK. Likely linked to the increase in adiponectin, the mice also showed an increase in energy expenditure. Further evidence suggests that GSK3 could be important for pancreatic endocrine function. Beta cell-specific expression of a constitutively active GSK3b transgene (GSK3b S9A) resulted in a reduction in beta cell mass

and function (Liu et al. 2008). Conversely, GSK3b deletion conferred resistance to high-fat-diet-induced diabetes and resulted in beta cell expansion in mice (Liu et al. 2010). Small-molecule inhibitors of GSK3 increased the replication of beta cells from isolated rat islets, suggesting that targeting GSK3 may have therapeutic value (Mussmann et al. 2007).These data show that GSK3 can impact multiple aspects of the metabolic syndrome, including insulin sensitivity, glucose clearance and storage, and lipid homeostasis. These studies highlight the distinction that needs to be made in terms of GSK3 protein levels and phosphorylation status, tissue specificity in GSK3 actions, and how its sensitivity to different signaling inputs not only allows for distinct outcomes of its activation but also means that context must be considered if GSK3 is to be a druggable target for metabolic dysfunction.

Alzheimer's disease The role of GSK3 as a mediator of Alzheimer's disease pathology has been studied on and off for a few decades. GSK3a and GSK3b isoforms can each phosphorylate residues on tau in vitro (Hanger et al. 1992); however, GSK3b but not GSK3a has been shown to co-localize with neurofibrillary tangles (NFTs) and active but not inactive (phosphorylated at S9) GSK3b was found in neurons in the early stages of tangle formation, suggesting that kinase active GSK3b is involved in the pathogenesis of NFTs (Yamaguchi et al. 1996; Pei et al. 1999). This is supported by genetic evidence showing that neuron-specific overexpression of GSK3b in mice resulted in hyperphosphorylation of tau on residues that lead to paired helical filament formation (Lucas et al. 2001). GSK3 has also been connected to amyloid plaque pathology. Lithium treatment blunted processing and secretion of amyloid precursor protein (APP) peptide fragments from cells and lowered levels of A-beta in the brains of a genetic model of Alzheimer's disease (Phiel et al. 2003). In cells, the effect of lithium to promote amyloid secretion was attributed to GSK3a, but not GSK3b. This finding is supported by in vivo evidence showing that GSK3a, but not GSK3b, knock-down ameliorates amyloid plaque load in a mouse model of Alzheimer's disease (Hurtado et al. 2012). This same study demonstrated that knock-down of both GSK3 isoforms reduced tau phosphorylation, misfolding, and memory deficit. GSK3 has also been implicated as a mediator of neuroinflammation, neuritic damage, and learning and memory deficits (Lucas et al. 2001; Hernandez et al. 2002). Mice with induced overexpression of GSK3b exhibit phosphorylation and mislocalization of tau, along with increased nuclear beta-catenin. These cellular phenotypes were associated with cognitive impairments and gliosis even in the absence of NFT deposition. GSK3b associated neuronal apoptosis, decreased brain volume, and impairment in learning in memory were completely reversible after 6 weeks of transgene silencing, pointing to a direct role of GSK3 in producing Alzheimer's disease-related traits (Engel et al. 2006). Additional studies have established that GSK3b promotes long-term depression and inhibits long-term potentiation, further connecting GSK3 to memory impairment (Hooper et al. 2007). More recently, GSK3 was shown to be activated in mouse models of Alzheimer's disease and inhibition was shown to attenuate dendritic spine loss (DaRocha-Souto et al. 2012). Mechanistically, the protective effect of GSK3 inhibition was linked to preserved CREB activity and increased expression of the CREB target gene BDNF. A recent clinical trial in patients who were already being treated for Alzheimer's disease suggests that disease progression and extent of GSK3 inhibition will both be important factors to consider in using GSK3 as a treatment target (Lovestone et al. 2015). Peptides that mimic GSK3-primed substrates are currently being developed and may provide advantages over ATP-competitive inhibitors, including higher selectivity and weaker GSK3 inhibition (Eldar-Finkelman and Martinez 2011). These studies and others convincingly show GSK3 as a player in Alzheimer's disease (Llorens-Marítin et al. 2014), but raise questions about the sequence of events in the etiology and progression of spontaneous age-related Alzheimer's disease. It will be of profound interest to understand which of the roles identified for GSK3 in Alzheimer disease models, as a factor promoting amyloid and NFT pathology or as a factor responding to the burden of amyloid and NFT pathology, is the more physiologically important one during human disease development and how it might be effectively targeted as a clinical intervention.

Cancer As a key regulator of cell fate, GSK3 has been implicated in the biology of many different cancers, both as an oncogene and as a tumor suppressor. Elevated GSK3b expression has been observed in pancreatic cancer cell lines, where it was responsible for promoting NF-kB activity and was necessary for NF-kB-mediated proliferation and survival (Ougolkov et al. 2005). High levels of GSK3 expression have been observed in colon,

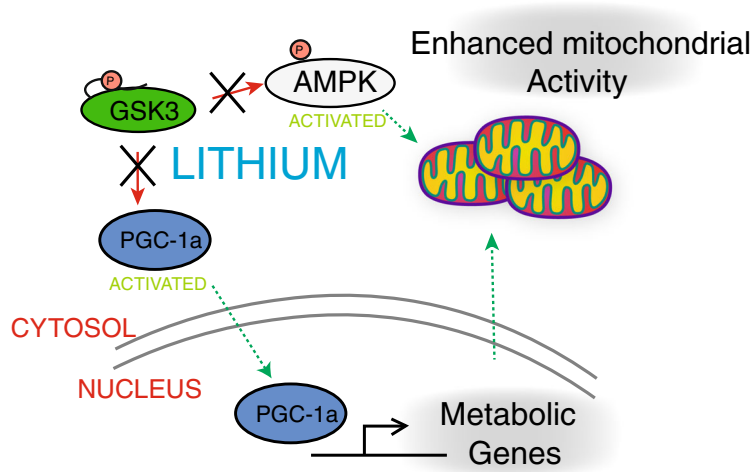

Fig. 5 GSK3b regulates mitochondrial energy metabolism. Inhibition of GSK3b by lithium stabilizes PGC1a and increases mitochondrial respiration. Activated PGC1a increases expression of target genes coincident with increased respiration. GSK3 negatively regulates AMPK and activating phosphorylation of AMPK is increased with lithium. These nutrient sensing pathways converge on mitochondrial function, suggesting that GSK3 is a key upstream regulator of metabolism

liver, and ovarian cancers; however, the physiological significance of this is not known (McCubrey et al. 2014). The role of GSK3 as a tumor suppressor is perhaps better understood. GSK3 is an effector of the WNT signaling pathway that targets beta-catenin for degradation, thus blocking the transcription of oncogenic targets. Expression of a kinase-inactive form of GSK3b in mice acts as a dominant negative and promotes the formation of mammary tumors that express high levels of beta-catenin and cyclin D1 (Farago et al. 2005). GSK3 may also play a role in the enhanced survival of cancer cells by controlling the localization and activation of bcl-2 family protein Bax, a key regulator of apoptosis (Maurer et al. 2014). Mcl-1 is another bcl-2 family protein linked to GSK3. Ablation of the GSK3b phosphorylation site on stabilizes Mcl-1, blocks apoptosis, and desensitizes cells to chemotherapeutics, again supporting a role for GSK3 in cancer development and as a target for enhancing cancer therapies (Ding et al. 2007). Another aspect of cancer biology linked to GSK3 is the epithelial-mesenchymal transition (EMT), a process that renders cancer cells more motile and metastatic. GSK3 targets the transcriptional repressor Snail for proteasomal degradation resulting in maintenance of E cadherin expression (Doble and Woodgett 2007). E cadherin expression is required for normal adhesion of epithelial cells, so loss of this protein is a key step in the EMT. Taken together, these studies reveal a complex and often contradictory role for GSK3 in the biology of cancer. Importantly, the stabilization of beta-catenin remains an unwanted consequence of GSK3 inhibitors. Development of isoform-selective inhibitors for GSK3 has promise for the treatment of acute myeloid leukemia (Wagner et al. 2018). In this study, selective inhibition of GSK3a was shown to block AML colony formation without causing beta-catenin stabilization, and impaired leukemia initiation and prolonged survival in vivo. Many GSK3 inhibitors fail clinical trials because of off-target toxicities. A better understanding of the biology of GSK3 will be imperative if treatments focusing on this key growth responsive kinase are to be selective and effective.

Normative aging The role of GSK3 in normative aging has only recently been investigated. GSK3b protein levels increase in multiple regions of the rat brain with age (Lee et al. 2006). Conversely, the neuroprotective intervention of CR is associated with lower protein levels of GSK3b in both mouse and rhesus hippocampus and relatively greater levels of inhibitory serine-9 phosphorylation (Martin et al. 2016). Further evidence for a role of GSK3 in aging comes from shorter lived species. In worms, GSK3 inhibition resulted in dose-dependent increases in lifespan that was accompanied by chromatin remodeling (McColl et al. 2008). Another study found that lithium increased mitochondrial energetics in worms and postulated that lithium resulted in selective autophagy of dysfunctional mitochondria (Tam et al. 2014). In flies, lithium resulted in dose-dependent reduction in triglycerides and increased

xenobiotic resistance, and the lifespan promoting effect of lithium was dependent upon activation of NRF-1 (Castillo-Quan et al. 2016). Conversely, overexpression of Shaggy, the GSK3 homolog in flies, resulted in shortened lifespan that was partially rescued by lithium. These results show that GSK3 is relevant to longevity and suggest that its ability regulate metabolism is central to its role in aging. Although GSK3b KO is embryonically lethal, GSK3a KO mice exhibit shortened lifespans and increased age-related pathology (Zhou et al. 2013). This includes cardiac dysfunction, early onset of sarcopenia, and increases in cellular senescence, some of which are thought to occur partially through activation of mTOR complex 1. Therefore, it appears that both GSK3 loss of function and gain of function can negatively impact lifespan under different circumstances. Given the established role of GSK3 in development, studies that use conditional modulation of GSK3 would be required to dissect its role specifically in the aging process.

Conclusion

From its initial identification as a regulator of glycogen metabolism, the known functions of GSK3 have expanded to encompass numerous fundamental pathways. GSK3 plays multifactorial roles in growth signaling, inflammation, senescence, cell fate, and energy metabolism. Each of these pathways has been implicated in age-related dysfunction, suggesting that GSK3 may play a central role in the increased disease vulnerability that accompanies aging. Many of the disease and disorders of age have been studied in isolation and many transgenic mouse studies employ young animals. Nonetheless, across the literature, there is an interesting case to be made for GSK3 as a driver in aging and even perhaps as a potential target for intervention. Evidence to date suggests that GSK3 actions and responsivity to perturbation are highly context-dependent: differences in interactions and flux through GSK3-regulated pathways occur as a function of cell type, tissue type, and background metabolic status. High granularity studies focusing on GSK3 biology, in particular the impact of status and combinations of signaling inputs on cellular outcome, should be highly informative. If dysregulation of GSK3 is indeed causal age-related disease vulnerability, targeted therapeutic strategies could prove effective across a spectrum of age-related diseases.

Acknowledgments The study was conducted with the use of resources and facilities at the William S. Middleton Memorial Veterans Hospital, Madison WI. The authors declare no conflict of interest.

Funding information This work was supported by NIH AG057408, The Glenn Foundation for Medical Research, the American Federation for Aging Research, and NIH training fellowship AG000213 (DSC).

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