Review Article

Deconstructing GSK-3: The Fine Regulation of Its Activity

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Glycogen synthase kinase-3 (GSK-3) unique position in modulating the function of a diverse series of proteins in combination with its association with a wide variety of human disorders has attracted significant attention to the protein both as a therapeutic target and as a means to understand the molecular basis of these disorders. GSK-3 is ubiquitously expressed and, unusually, constitutively active in resting, unstimulated cells. In mammals, GSK-3α and β are each expressed widely at both the RNA and protein levels although some tissues show preferential levels of some of the two proteins. Neither gene appears to be acutely regulated at the transcriptional level, whereas the proteins are controlled posttranslationally, largely through protein-protein interactions or by posttranslational regulation. Control of GSK-3 activity thus occurs by complex mechanisms that are each dependent upon specific signalling pathways. Furthermore, GSK-3 appears to be a cellular nexus, integrating several signalling systems, including several second messengers and a wide selection of cellular stimulants. This paper will focus on the different ways to control GSK-3 activity (phosphorylation, protein complex formation, truncation, subcellular localization, etc.), the main signalling pathways involved in its control, and its pathological deregulation.

1. Introduction

Glycogen synthase kinase-3 (GSK-3) is a CMGC serine/threonine protein kinase initially described as one of the kinases that phosphorylates and inhibits glycogen synthase [1]. It is now widely accepted though that GSK-3 plays an important role in various essential physiological processes, such as development, cell cycle, or apoptosis [2]. Apart from glycogen synthase, a plethora of different substrates has been identified in all cellular compartments, that is, metabolic proteins [3], cytoskeletal proteins [4], and transduction [5] and transcription factors [6] (see Table 1).

In neuronal development, GSK-3 has been reported to control morphogenesis and axonal polarity [7], synaptogenesis [8], and survival [9, 10]. In addition, GSK-3 dysfunction has been associated with brain pathological conditions, such as Alzheimer's disease (AD) [11, 12] or prion neurotoxicity [13]. Thus, the deep knowledge of the role of both GSK-3 isoforms in brain metabolism will allow us to understand their contribution to neurodegenerative processes.

GSK-3 unique position in modulating the function of a diverse series of proteins in combination with its association with a wide variety of human disorders has attracted significant attention to the protein both as a therapeutic target and as a means to understand the molecular bases of these disorders. Furthermore, GSK-3 appears to be a cellular nexus, integrating several signalling systems, including numerous second messengers and a wide selection of cellular stimulants.

2. GSK-3 Structure

GSK-3 has been highly conserved during evolution, and homolog genes have been identified in virtually every eukaryotic genome investigated, including species, such as *Dictyostelium discoideum*, *Xenopus laevis*, or *Drosophila melanogaster* [14–16]. In mammals, GSK-3 is encoded by two genes known as *gsk-3α* and *gsk-3β* [17, 18] encoding GSK-3α (483 *aa* in humans) and GSK-3β (433 *aa*) proteins with apparent molecular masses of 51 and 47 kDa, respectively. Both isoforms are almost identical (98%) within their ATP binding pocket but differ at their N- and C-terminal domains [19]. A neuron-specific splicing isoform (β2) having an

TABLE 1: GSK-3 substrates.

Metabolic proteins	Structural proteins	Transcription factors
Glycogen synthase	Tau	AP-1
ATP cytrate lyase	MAP1B	β-catenin
PKA	MAP2	CREB
PDH	NCAM	C/EBP
Acetyl-CoA carboxylase	Neurofilaments	Myc
PP1	CRMP2	NFκB
PP2A inhibitor	Dynein	NFAT
PP2A	Dynamin-like protein	GR
Cyclin D1	MBP	HSF-1
eIF2B	APC	Notch
NGF receptor	Kinesin light chain	p53
Axin		HIF-1
APP		
Bax		
VDAC		
Hexokinase		
Presenilin		
LRP5/6		

GSK3 Selectivity
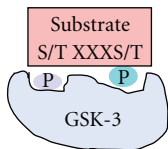
insertion of 13 *aa* within the substrate-binding domain has also been described [20]. Mammalian GSK-3α and β are each widely expressed although some tissues show preferential levels of some of the two proteins. Neither gene appears to be acutely regulated at the transcriptional level.

Crystallographic studies have revealed the three-dimensional structure of GSK-3β [21, 22]. Its overall shape is shared by all kinases, with a small N-terminal lobe mostly consisting of β-sheets and a large C-terminal lobe essentially formed of α-helices [23]. The ATP binding pocket is located between the two lobes and although, is well conserved among kinases [24], it is possible to obtain selective inhibitors by taking advantage of the small differences that exist between the different kinases. Current availability of crystal structures of complexes of GSK-3β with a variety of ligands, together with molecular modelling approaches, provides the necessary clues for enhancing selectivity towards GSK-3 [22].

Some GSK-3 substrates do not require a very specific sequence, but rather a previous (*primed*) phosphorylation by a *priming* kinase on a Ser or Thr residue located four aminoacids, C-terminal to the Ser or Thr residue to be modified by GSK-3 (see below for regulation through primed phosphorylation). The crystal structure of human GSK-3β has provided a model for the binding of prephosphorylated substrates to the kinase. According to it, *primed* Ser/Thr is recognized by a positively charged binding pocket formed by residues Arg96, Arg180, and Lys205 that facilitates the binding of the phosphate group of primed substrates. GSK3β uses the phosphorylated serine or threonine at position +4 of the substrate to align of the two domains for optimal catalytic activity [21, 22].

Furthermore, crystal structures of GSK-3β complexes with interacting proteins FRAT/GBP and axin have allowed defining the molecular basis for those interactions, which play critical role in some signalling pathways (see below for regulation through protein complex formation). These studies confirm the partial overlap of the binding sites of axin and FRAT1/GBP predicted from genetic and biochemical studies [2, 25] but reveal significant differences in the detailed interactions and identify key residues mediating the differential interaction with both proteins. This ability of GSK-3β to bind two different proteins with high specificity *via* the same binding site is mediated by the conformational plasticity of the 285–299 loop, while some residues in this versatile binding site are involved in interactions with both axin and FRAT; others are involved uniquely with one or the other [26].

3. How Is GSK-3 Activity Controlled?

As already mentioned above, one of the main characteristics of GSK-3 is that its activity is high in resting, unstimulated cells while regulated by extracellular signals that typically induce a rapid and reversible decrease in enzymatic activity. Glycogen synthase kinase-3 is a dual specificity kinase differentially regulated by tyrosine and serine/threonine phosphorylation [27]. And for many years, it was believed to be a constitutively active kinase; however, it has become apparent that the activity of GSK-3 may be regulated by a variety of means. In fact, control of GSK-3 activity occurs by complex mechanisms that are each dependent upon specific signalling pathways. Thus, the regulatory mechanisms can be classified as follow.

3.1. Regulation by Phosphorylation. The first regulatory mechanism described of GSK-3 activity involved the phosphorylation of specific residues of GSK-3 by other kinases, and more recently through autophosphorylation [17, 28].

Four different regions and residues have been described in the GSK-3 molecule. The first one corresponds with a serine residue at positions 21 in GSK-3α and 9 in GSK-3β. It has been clearly established that phosphorylation of serine 21 or 9 correlates with the inhibition of its kinase activity [29–31]. Many protein kinases are capable of phosphorylating GSK-3 at this residue, such as Akt, ILK, PKA, and p90Rsk [32, 33], and many physiological situations of inhibition of GSK-3 correlate with serine phosphorylation, such as Insulin/IGF1, NGF, or estradiol treatments, not only in neurons [34].

Additionally, two other regulatory sites have been described. One is the threonine 43, present only in the isoform GSK-3β, which may be phosphorylated by Erk [35].
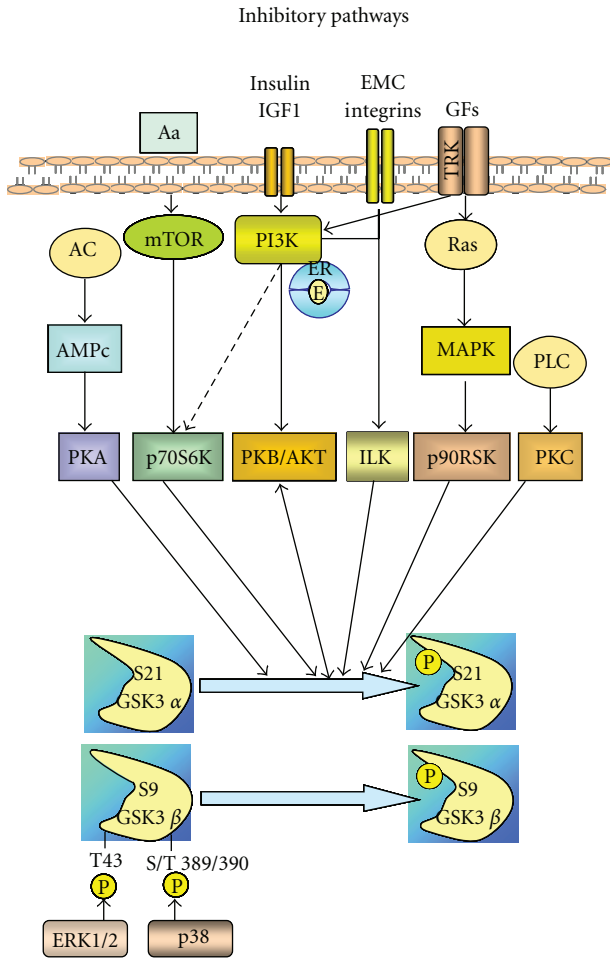
Inhibitory pathways

FIGURE 1

Activation signaling

FIGURE 2

This phosphorylation correlated with GSK-3 inhibition. Second, serine 389 and threonine 390 present in GSK-3β have been shown to be phosphorylated by p38 MAPK [36]. In both cases, the data suggested that this phosphorylation may increase the capacity of Ser-9 to be phosphorylated rather than promote a direct inhibition (see Figure 1).

In contrast, tyrosine phosphorylation present in positions 279 in GSK-3α or 216 in GSK-3β, appears to correlate with an increase of its kinase activity [37]. Different candidates such as Pyk-2 and Fyn kinases have been reported to be able to phosphorylate GSK-3 *in vitro* on tyrosine. In addition, MEK1/2 has been showed to have this capacity only in fibroblasts [38, 39]. This data contrast with those reported in *Dictyostelium discoideum* where there is compelling evidence indicating that ZAK 1 is responsible for generating tyrosine phosphorylation in GSK-3 [14, 40]. However, no homologue of such kinase has been found in mammals.

More recently, an alternative hypothesis has been proposed for the regulation of GSK-3 tyrosine phosphorylation. This hypothesis suggests that in mammalian systems phosphotyrosine in GSK-3 corresponds to an intramolecular autophosphorylation event and may be regulated by Hsp90 [41]. Molecular dynamics and crystallographic
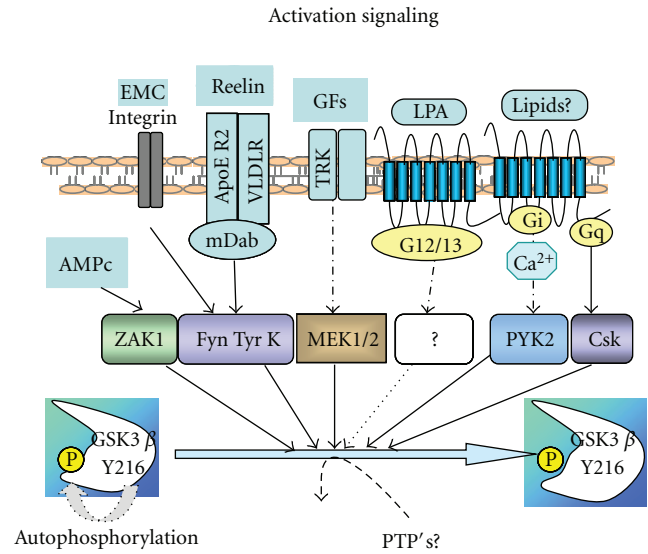
studies clearly suggest that Tyr216 renders the kinase active through interactions with Arg220 and Arg223, stabilizing the activation loop and allowing full substrate accessibility [42, 43]. However, this hypothesis still lacks a cellular demonstration.

However, our data indicated that not all pharmacological inhibitors of GSK-3 decrease the level of phosphotyrosine. Therefore, lithium chloride inhibits GSK-3 activity, but this inhibition does not alter its pTyr content [44]. Moreover, in neuronal cells, tyrosine phosphorylation of residue 216 or 279 increased following exposure to LPA [37] and even upon exposure of neurons to β-amyloid or PrP [13, 45, 46] in a clear correlation with an increase in GSK-3 activity. In addition, in many neuronal cells, the pharmacological inhibition of tyrosine phosphatases with *ortho*-vanadate increases the basal level of GSK-3-pTyr [44]. Thus, considering all these data, in addition to this tantalizing autoregulatory system proposed, we hypothesized that some as-yet-unidentified tyrosine kinases and phosphatases may also regulate this kinase (see Figure 2).

3.2. Regulation by Protein Complex Association. One regulatory mechanism that is still not fully understood involves the interaction of the GSK-3 with structural proteins (scaffold proteins). It is well known that GSK-3 contributes to a multiprotein complex formed by axin and adenomatous polyposis coli (APC), among others (for review see, i.e., [47]). This protein complex is the core of canonical Wnt signalling (see below). Indeed, in the absence of ligand, GSK-3 is able to phosphorylate β-catenin for targeting it for proteasome degradation [48]. More recently, some data suggests that this complex may be specific for GSK-3β2 isoform [49], which opens the possibility of a deeper analysis of "specific functions of GSK-3 isoforms."

Another system of protein-kinase interaction was denoted as GSK-3-binding protein (GBP or FRAT) [25, 50].

Three different FRATs have been cloned and characterized; however, their mechanism of action is not well understood. FRAT1 appears to act as an inhibitory system [51], whereas FRAT2 appears to preferentially increase GSK-3-mediated phosphorylation in some residues [52]. Surprisingly, recent data demonstrated that FRAT is dispensable because the triple FRAT-knockout mouse lacks any major defect in brain development [53]. All these data indicated that the precise role of FRAT in GSK-3 regulation is still to be defined.

Using the binding site on GSK-3 for FRAT/GBP, a GSK-3-interacting protein symbolized by GSKIP has been cloned and characterized. GSKIP can block phosphorylation of different substrates and functions as a negative regulator of GSK-3 beta [54].

3.3. Regulation by Priming/Substrate Specificity. As previously mentioned, the specificity of many kinases is governed by a consensus sequence of aminoacids sequence. However, as almost general rule, GSK-3 substrates do not require a very specific sequence, but a previous (*primed*) phosphorylation residue modified by a *priming* kinase located four aminoacids, C-terminal, to the Ser or Thr residue to be modified by GSK-3. The crystal structure of human GSK-3β provides a model for the binding of prephosphorylated substrates to the kinase (PDB ID are 1I09 [22] and 1H8F [21]). According to it, *primed* Ser/Thr is recognized by a positively charged binding pocket formed by residues Arg96, Arg180, and Lys205 that facilitates the binding of the phosphate group of primed substrates.

Some "priming kinases" have been identified, such as cdk-5 [55–57], PAR-1 [58], casein kinase I [59], PK-C [60], or PK-A [57]. However, it is not clear so far whether a second set of "*nonprimed*" substrates may define a different group of functions [61]. In addition, different glycogen synthase kinase-3 isoforms appear to exhibit distinct substrate preference in the brain [62].

3.4. Regulation by Subcellular Localization. In developmental brain, the presence of GSK-3 was high at E18 and peaked on P8, decreasing after that period [63]. In addition, this report showed that the developmental profile of GSK-3α and GSK-3β is different, having β downregulated after birth which suggested a differential role in neuronal development. However, the putative differential role of each isoform has been explored in few reports, that is, [7]. It is important to indicate that a portion of GSK-3, mostly β, has been reported to be associated in the growth cone. This GSK-3 pool appears to respond rapidly, being modified by phosphorylation and/or relocated in the growth cone by external signals such as Semaphorins [64] or NGF [65].

GSK-3 activity is also dependent on its subcellular localization; some data illustrated the presence of GSK-3α and β in many neuronal compartments and in primary neurons, either in axon, dendrite, or in nucleus [66, 67]. In addition, GSK-3 has been found in the cytoplasm, nucleus and the mitochondria [18]. Considering the list of GSK-3 substrates reported, it is evident that most of its activity should occur in the cytoplasm and in the nucleus, while we

have less information about GSK-3 potential targets in the mitochondria. Recent data suggested that proteins such as Mcl-1 [68] and hexokinase [69] may be regulated by GSK-3 activity. It has been suggested that nuclear GSK-3 may be involved in phosphorylation of many transcription factors such as cyclin D1, β-catenin, HSF-1, NFAT, and cAMP-response element-binding protein, among others (Table 1), for review see [28, 70, 71]. Also, it has been proposed that GSK-3 in the nucleus may have a role in alternative splicing [72]. In addition, proapoptotic stimuli induce nuclear accumulation of GSK-3β [73]; however, this hypothesis has been not established in other neuronal death paradigms (D. Simon, unpublished observations).

Further insight into GSK-3 regulation has been gained very recently by revealing an essential role of multivesicular endosomes in the Wnt signalling pathway. A combination of protease protection assays, detergent permeabilization, and cryoimmunoelectron microscopy demonstrated that Wnt activation of the Frizzled and LRP6 receptors triggers sequestration of GSK-3 into these membrane-bounded organelles, leading to decreased GSK-3 levels in the cytosol [74]. This process seems to require β-catenin, forming a feed-forward loop by facilitating GSK-3 sequestration.

3.5. Regulation by Proteolytic Cleavage. A new mechanism of GSK-3 regulation has been recently proposed. This regulation involves the removal by calpain of a fragment from the N-terminal region of GSK-3, including the regulatory serines 9/21. After removal of that fragment, GSK-3 becomes activated [75]. The same study showed that both isoforms α and β are cleaved by calpain, although with different susceptibility. Moreover, GSK-3 truncation has been observed in human and mouse postmortem brain tissue [76]. It is noteworthy to consider that a similar mechanism has been described for β-catenin in hippocampal neurons, where after NMDA-receptor-dependent activation, calpain induced the cleavage of β-catenin at the N terminus, generating stable and truncated forms which maintain its transcriptional capacity [77]. Likewise, GSK-3 truncation is mediated by extracellular calcium and can be inhibited by memantine [76], an NMDA antagonist used for the treatment of Alzheimer's disease. Interestingly, GSK-3β has also been recently shown to be cleaved at the N-terminus (and subsequently activated) by matrix metalloproteinase-2 (MMP-2) in cardiomyoblasts [78].

4. Pathways Controlling GSK-3 Activity in Neurons

The regulation of GSK-3, as previously mentioned, is an essential regulatory key controlling many physiological processes in neurons. Many external signals may trigger pathways that finally may activate or inhibit GSK-3 activity, either transiently or in more sustained way. These "physiological pathways" could be subdivided in two major clusters, those that essentially have to inhibit GSK-3 activity, and second, those that may, at least transiently, trigger GSK-3 activity.

4.1. Inhibitory Pathways. Among these pathways, the signalling triggered by Insulin or IGF-1 [19, 79, 80] and NGF/BDNF/NT3 [81, 82] has similar features. These tyrosine kinase receptors initiated cytoplasm signals in which the inhibition of GSK-3 activity is a common feature. It is generally accepted that the kinase implicated in this inhibition is PKB/Akt [29–31], even though kinases such as PKA or ILK have also been implicated [32, 33]. In all cases, phosphorylation on serine 21 and 9 (α and β, resp.) represents the inhibition of the GSK3 kinase activity, as previously mentioned.

The second well-documented pathway is Wnt/Wingless signalling [83, 84]. This signalling has been widely studied, and it has been shown to be essential in early embryonic patterning, cell fate, cellular polarity, and cell movement in both vertebrates and invertebrates [47, 85]. In many if not all cell systems, the canonical Wnt pathway is formed by a set of phylogenetically conserved proteins including the Wnt receptor *frizzled* (*fz*), and a coreceptor LRP5/6; *Dishevelled* (*Dsh*), and a scaffolding protein that activates a complex formed by Axin/APC/GSK-3-β/β-catenin [47, 85–88].

In this pathway, in the absence of ligand, GSK-3β phosphorylates β-catenin, among other proteins, this phosphorylation constituting part of a degradation signal for β-catenin. However, in the presence of Wnt, the receptor complex triggers a signal in which *Dsh* inhibits the activity of GSK-3β by a mechanism not completely understood, so far. This system appears to be specific for GSK-3β as no counterpart has been described for GSK-3α to date; however, some GSK-3 activity appears to be necessary for Wnt signalling [89, 90]. More recently, a bioinformatics-based screen for proteins whose stability may be controlled by GSK-3 [91] has led to the identification of a number of multiple Wnt signalling target proteins, suggesting that this pathway controls a broad range of cellular activities apart form β-catenin-mediated transcriptional activation. Furthermore, GSK-3-mediated Wnt signalling seems to regulate the turnover of many cellular proteins [74, 91], indicating that GSK-3 phosphorylation-dependent protein degradation may be a widespread cellular mechanism to regulate a variety of cellular processes in response to extracellular signals [71].

Functional segregation of the insulin/growth factor and Wnt roles requires either that there be no exchange between the subsets of the cellular GSK-3β pool committed to each role, or that the recruitment of GSK-3β to the axin-APC complex can reverse or override inhibitory Ser9 phosphorylation present in a recruited GSK-3β molecule. Phosphatases capable of removing extant Ser9 phosphorylation are certainly known to be associated with the axin-APC complex [92, 93]. Alternatively, the very substantial enhancement in activity towards β-catenin afforded by the axin "scaffolding" may simply allow a primed β-catenin substrate to outcompete a pSer9-GSK-3β N-terminal peptide for access to the substrate-binding site [26].

Estrogens regulate many physiological processes and fulfil a wide range of functions during development and differentiation in mammals of both sexes. The actions of estrogens are mediated by estrogen receptors and have been classified as either "genomic actions" or "nongenomic, rapid actions." The genomic actions are based on the capacity of the estrogen receptors (ERs) to modulate transcriptional activity either directly or through coactivators or corepressors, that is, [94, 95].

More recently, it has been shown that in addition to its direct transcriptional activity, estrogen receptors activate a set of cytoplasm signals in a similar manner to some growth factors. Hence, it has been reported that estradiol acts synergistically with IGF-1 in the brain or in neurons, activating the PI3K/Akt pathway [34, 96, 97]. We described that the addition of estradiol increases the serine phosphorylation of GSK-3. This inhibitory phosphorylation is time- and concentration dependent, and an antagonist of estradiol prevents this event. The kinase responsible is sensitive to the inhibition of the PI3K pathway, and for this reason, it seems that the best candidate would be Akt [98, 99]. A more detailed analysis of these new signals will give us clear evidence whether this pathway is completely convergent with those using PI3K/Akt/GSK3, as previously mentioned.

4.2. Activation Pathways. In neurons, LPA has been shown to induce neurite retraction and the rounding up of neuroblastoma cell lines [100]. In some primary neurons, it also promotes growth cone collapse and neurite retraction [37, 101]. This bioactive lipid acts as a growth factor through specific seven transmembrane domain receptors, denoted as *lpa* 1–4 [102, 103]. We described that GSK3 activity was increased after LPA treatment in diverse neuronal cells of different species in correlation with the neurite retraction process [104, 105]. This activation correlated with an increase in GSK3-PTyr and may be downstream $G\alpha_{12}$ or $G\alpha_{13}$ [101, 105, 106]. The previous inhibition of GSK3 activity prevents, at least in part, the growth cone collapse response. Similarly, it has been reported that three different GSK-3 antagonists (LiCl, SB-216763, and SB-415286) can inhibit the growth cone collapse response induced by Sema 3A [64].

However, the exact mechanism of how this activation of GSK3 occurs is not known, so far. Many reports indicate that in *Dictyostelium discoideum* GSK-3 activity may increase in response to cAMP binding to a heptahelical G-protein-coupled receptor. In this system, a tyrosine kinase and a tyrosine phosphatase have been described as regulators of GSK-3 activity [14, 40], but similar kinase and phosphatase have not been found in mammals.

Furthermore, it has been reported that Reelin and Netrin increased GSK-3 activity, similar to what LPA did. This Netrin or Reelin-dependent GSK-3 activation seems not to be a particular characteristic of the cell line or neuron used but rather a more general physiological process [107–109]. Indeed, even in situations where the final balance is an inhibition of GSK-3 kinase activity, such as following the addition of IGF1/Insulin or after estradiol addition, a transient activation of GSK-3 could be observed [34, 38]. All these data suggest that the upregulation and downregulation of this kinase is more complex than might initially have been considered.

5. Pathological Activation of GSK-3

Deregulation of GSK-3 has been linked to a wide range of human pathological conditions including type II diabetes, muscle wasting, cancer and neurological disorders such as bipolar disorder, schizophrenia, depression, stroke, sleep disorders, and Alzheimer's disease (AD), among others, for a review see [110].

Lithium and valproic acid are mood stabilizers widely used in the chronic treatment of bipolar disorders. Lithium ions directly inhibit GSK-3 [111], most likely by competing with magnesium [112], while valproic acid is able to inhibit GSK-3 activity in relevant therapeutic concentrations in human neuroblastoma cells [113] although *in vivo* direct inhibition of GSK-3 by valproic acid remains a matter of debate [114]. The precise mechanism of action by which lithium exerts its therapeutic effects is not known, but it is conceivable that the acute effects on GSK-3 result in changes in gene regulation and cellular changes which could affect the neuronal plasticity over time [115]. Actually, lithium is also an inhibitor of several phosphomonoesterases [116] and phosphoglucomutases [117], but the fact that GSK-3 has been shown to be significantly inhibited at therapeutic lithium concentrations [118–120] suggests that at least a significant proportion of lithium's therapeutic actions in bipolar disorder results from the inhibition of GSK-3, underlying its importance as a therapeutic target for this disorder [121, 122].

Lymphocytes of patients with schizophrenia show impaired GSK-3 protein levels and activity [123], whereas GSK-3 has been reported to be reduced in the frontal cortex of postmortem schizophrenic brains [124]. Since the Wnt family of genes plays a central role in normal brain development, it is possible that GSK-3 impairment may lead to abnormal neuronal development. More recently, a direct association has been shown between GSK-3 and the N-terminal region of disrupted-in-schizophrenia-1 (DISC1), a strong genetic risk factor associated with schizophrenia [125]. Moreover, mounting evidence suggests that GSK-3 is a crucial node that mediates various cellular processes that are controlled by multiple signalling molecules such as DISC-1, PAR3, PAR6, and Wnt proteins that regulate neurodevelopment [88].

Interestingly, increased levels of GSK-3 have also been reported in postmortem analysis of brains from AD patients compared to age-matched control samples [126], whereas a spatial and temporal pattern of increased active GSK-3β expression correlates with the progression of NFT and neurodegeneration [127]. Apart from being the major kinase to phosphorylate tau both *in vitro* and *in vivo* [128], GSK-3 has been recently proposed as the link between the two major histopathological hallmarks of AD, the extracellular amyloid plaques and the intracellular NFT [129, 130].

Exposure of primary neuronal cultures to Aβ induces activation of GSK-3 [131], tau phosphorylation [132], and cell death [133, 134], whereas blockade of GSK-3 expression by antisense oligonucleotides or its activity by lithium inhibits Aβ-induced toxicity [135, 136]. GSK-3 inhibition *per se* decreases Aβ production in cells and in an animal

model of amyloidosis [61, 120], most likely through a mechanism involving inhibition of γ-secretase [137]. Furthermore, amyloid precursor protein (APP) itself is a substrate for GSK-3 *in vitro* and *in vivo* [138]. Finally, modulation of the GSK-3 signalling pathway by chronic lithium treatment of transgenic animals might also have neuroprotective effects by regulating APP maturation and processing [138].

In tauopathies such as frontotemporal dementia with Parkinsonism (FTDP) linked to chromosome 17, the presence of some mutations in tau protein correlates with the onset of the disease [139, 140]. Treatment of transgenic mice overexpressing mutant human tau (P301L, 4RON), with the GSK-3 inhibitor lithium, has been shown to significantly decrease the levels of tau phosphorylation and significantly reduce the levels of aggregated, insoluble tau. Administration in this model of a second GSK-3 inhibitor, AR-A014418, also correlated with reduced insoluble tau levels, supporting the notion that lithium exerts its effect through GSK-3 inhibition [23]. More recently, chronic lithium administration has also been shown to reduce tau phosphorylation in the 3xTg-AD mice [141], but did not significantly alter the amyloid load.

An increase in GSK-3 activity has also been shown to coincide with cell death following middle cerebral artery occlusion in mice which results in cortical infarcts [142], and a reduction in infarct volume with the GSK-3 inhibitor lithium was demonstrated [143], indicating that GSK-3 inhibition may be beneficial in stroke. In fact, pharmacological inhibition of GSK-3 reduced infarct volume and improved behaviour in a focal cerebral ischemia model [144].

6. GSK-3 as a Therapeutic Target

Besides deregulation of its activity in neurodegenerative processes, mounting evidence further suggests a potential role for GSK-3 as a therapeutic target in a range or other pathologies, including pancreatic cancer [145], parenchymal renal diseases [146], and HIV-1-associated dementia [147], among others.

The recent discovery that glycogen synthase kinase-3 (GSK-3) promotes inflammation through nuclear factor *kappa* B (NFκB) has revealed new functions on regulating inflammatory processes [148]. Furthermore, GSK-3 inhibition provides protection from inflammatory conditions in different animal model [149], suggesting that GSK-3 inhibitors may have multiple effects influencing these conditions.

Finally, recent developments suggest an active role of GSK-3β in various human cancers, although its role in tumourigenesis and cancer progression remains controversial. It may function as a "tumour suppressor" for certain types of tumours, whereas it seems to promote growth and development for some others. Deregulation of GSK-3β has been shown to promote gastrointestinal, pancreatic, and liver cancers and glioblastomas. Furthermore, GSK-3β inhibition attenuates cell survival and proliferation, induces cell senescence and apoptosis, and sensitizes tumour cells to chemotherapeutic agents [150] and ionizing radiation [151]. Nevertheless, an attractive target for a variety of human

diseases, its therapeutic potential on tumourigenesis, and cancer chemotherapy still needs to be carefully evaluated [152].

The close involvement of GSK-3 activity in different human pathologies has sparked intense efforts in developing inhibitors as therapeutic agents. Thus, the discovery of small molecule GSK-3 inhibitors in the last few years has not only attracted significant attention to the protein as a therapeutic target but also has provided a means to further understand the physiological functions of GSK-3 and to gain further insight into the molecular basis of those disorders.

In fact, at least one small molecule GSK-3 inhibitor program has made it to the clinic [153]. Tideglusib (NP-12) is a synthetic small molecule form the TDZD chemical class [110] which is currently in phase II development for two CNS indications: Alzheimer's disease and progressive supranuclear palsy (PSP), a tauopathy [154].

7. Conclusion

Three decades after its discovery as a protein kinase involved in glycogen metabolism, GSK-3 was revealed as a key enzyme in regulating many critical cellular processes, providing a link between many different substrates and various signalling pathways as well as gene expression. Modulation of its activity has also turned out to be much more complex than originally thought, as evident from what has been reviewed here. Furthermore, its role in a variety of highly relevant human pathological conditions has drawn significant attention to this enzyme as a potential therapeutic target, and the recent development of specific inhibitors has granted us new tools to dissect out its molecular and physiological functions while providing novel therapeutic agents. Taken all together, the next few years will certainly bring us further insights into the cellular functions of this fascinating enzyme.

Acknowledgments

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