GSK3

Glycogen synthase kinase-3 (GSK3) is a serine/threonine kinase. It was initially identified as a regulator (inhibitor) of glycogen synthesis. It has since been recognized as a multifunctional kinase with a variety of roles both in invertebrates and in vertebrate cells.

Keywords: glycogen synthase kinase-3 ; actin ; tubulin ; cytoskeleton ; brain development ; cancer cells migration ; mitochondria trafficking

1. Introduction

Glycogen synthase kinase-3 (GSK3) is a serine/threonine kinase. It was initially identified as a regulator (inhibitor) of glycogen synthesis [1]. It has since been recognized as a multifunctional kinase with a variety of roles both in invertebrates and in vertebrate cells. In mammals (humans included), there are two closely related isoforms of the kinase: GSK3α and GSK3β [2]. They share ~98% identity in their kinase domains, but they have distinct substrate preferences, and their cellular functions are, at least partially, non-redundant [3][4]. The β isoform predominates in the majority of cells, and it is also more studied. GSK3 requires phosphorylation on tyrosine 216 (β isoform) or 279 (α isoform) for maximal activity. As a constitutively active kinase, it is oftentimes inhibited in response to upstream signals by phosphorylation of S9 (GSK3β) or S21 (GSK3βα). As a sensor of growth factors (e.g., insulin, transforming growth factor-β, epidermal growth factor, nerve growth factor, and brain-derived neurotropic factor) and other extracellular stimuli GSK3 is a master switch kinase regulating various aspects of cellular function such as growth, repair, mobility, and survival. Therefore, it is not surprising that dysregulation of GSK3 activity is observed in many pathophysiological processes, including the development of cancer, and neurodegenerative and psychiatric disorders (for review see [5][6]). This also makes GSK3 an attractive therapeutic target, and intensive efforts have been undertaken to discover clinically relevant selective GSK3 inhibitors [7]. However, the pleiotropic functions of the kinase pose major obstacles in developing effective treatments without adverse effects.

In the still-increasing list of GSK3 substrates, there are, among others, proteins engaged in the regulation of actin cytoskeleton dynamics (e.g., Rho family members and related GTPases), microtubule-associated proteins (MAPs, e.g., Tau and collapsin response mediator protein 2 (CRMP 2)) and adhesion of cells to extracellular matrix (e.g., focal adhesion kinase, FAK) [8][9][10]. Acting through these substrates, GSK3 can influence cell polarization and directional migration, as well as intracellular trafficking of mitochondria and vesicular structures. The migration of cells is a fundamental process, especially important in the development of an organism. Although not all differentiated adult cell types migrate in vivo, almost all of them exhibit some form of spatial segregation of structures and functions and require some form of organelles' trafficking. This, in turn, requires the orchestration of activities of several signaling pathways. Since GSK3 lies at the crossroads of these pathways, it can be viewed as one of the coordinators/integrators of complex processes of cellular dynamics.

2. GSK3–Cytoskeleton Interplay in Brain Development and Pathology

GSK3β is expressed in all tissues, including the brain [2]. In the rodent central nervous system (CNS), GSK3β is more abundantly expressed than GSK3α, and thus, it is also better studied. This isoform is involved in numerous events during neurogenesis, synaptic plasticity, and neurodegeneration (for review see [6][11]). During brain development, GSK3β is highly expressed in neurons and barely detectable in astrocytes [12][13]. In rodent embryos, GSK3β is detected in axons, perikarya, and the proximal part of dendrites of postmitotic neurons (in neuroblasts, the kinase is hardly detectable), but after the 10th day of postnatal life, it starts to disappear from the axonal tracts. Globally, the expression of GSK3β in the brain is supposed to be higher in rodent embryos and in early postnatal life, than in adults, positively correlating with the major period of dendritic extension and synaptogenesis [12], although it has been suggested that in the murine brain (hippocampus, cerebral cortex, and cerebellum), such age-related changes applied to proteins comprising the "GSK3

proteome" rather than the GSK3 (α and β) protein level [14]. The expression of GSK3β increases again later in aged (24–29-month-old) rodent brains [15]. This increase is correlated with the development of neurodegenerative diseases such as Alzheimer's disease (AD) and thus is a promising target of a future anti-neurodegenerative therapy.

Substrates of GSK3β can be divided into three groups: metabolic/signaling proteins (e.g., acetyl-CoA carboxylase, pyruvate dehydrogenase, glycogen synthase, insulin receptor substrate-1, amyloid-beta precursor protein, cyclin D1, protein phosphatase 1); structural proteins (e.g., dynamin-like proteins, microtubule-associated protein 1B and 2 (MAP1B and MAP2), neural cell-adhesion protein, neurofilaments, spindle-associated protein Astrin, and microtubule-associated protein Tau), and transcription factors (e.g., activator protein 1 (AP-1), cAMP response element-binding protein, glucocorticoid receptor, Myc, nuclear factor of activated T cells and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)) [11]. Due to the long list of substrates, GSK3β can modulate numerous signaling pathways both directly and indirectly, leading to cytoskeleton remodeling. In turn, proper cytoskeleton maintenance and remodeling are indispensable for the correct development and functioning of the CNS.

Certain polarity-inducing signals have inhibited GSK3β and allowed MT stabilization and axon elongation [16][17][18]. However, it has been shown that the reduction of GSK3 activity should be "localized" and restricted to the axonal growth cone. Global inhibition of GSK3 caused axonal growth difficulties because of a reduced pool of dynamic microtubules at the growth cone that led to excessive MT stability [19]. Finally, after the axon elongates enough, it starts branching to innervate multiple targets. Once again, a proper level of GSK3β activity reduction (creating a kind of "intermediate conditions" that preserve a pool of dynamic microtubules) is needed for this process [19]. C-Type Natriuretic Peptide, cGMP, and cGMP-dependent protein kinase G1 are known to inhibit GSK3β activity at this point [20][21], but Wnt signaling also seems to be involved [22]. Additionally, JNK-interaction protein 3 (JIP3) can restrict axonal branching by interaction with GSK3β. Knocking down JIP3 or GSK3β increases axon branching [23]. Furthermore, PTEN, which dephosphorylates phosphatidylinositol (3,4,5)-triphosphate (PIP3) and decreases PI3K signaling is known to negatively regulate axonal branching. It has been proposed that during induction of the branching, downregulation of PTEN was caused by GSK3β inactivation [24][25]. Similar to axonal elongation, during axonal branching, the F-actin reorganization and MT extension occur [26]. However, it is not fully understood how molecular regulation of GSK3β is translated into axonal branching mechanisms, although it is known that local inactivation of GSK3β along the axon promotes cytoskeletal reorganization required for the process.

GSK3β can phosphorylate several proteins involved in cytoskeleton reorganization needed for morphological changes in synapses (synaptic plasticity) during memory consolidation. Due to synaptic plasticity, the connections between two neurons are either strengthened (in the process of long-term potentiation, LTP) or weakened (long-term depression, LTD) in response to increases or decreases of neuronal activity. Synaptic plasticity is a prerequisite to learning. The connection between two neurons can be strengthened by the recruitment of neurotransmitters' receptors to the membrane of the postsynaptic neuron [27] or by releasing higher amounts of neurotransmitters to the synaptic cleft by the presynaptic neuron [28]. In turn, the connection is weakened by retraction of the receptors and decreased neurotransmitter secretion. Some cytoskeletal changes occur during dendritic spine maturation. During strengthening of the connections, dendritic spines start to grow in volume creating mushroom-shaped outgrowth, the spines can also branch. This leads to increased surface area in the postsynaptic membrane, creating the possibility to incorporate more receptors into the membrane [29]. It has been demonstrated that during the connection strengthening, dendritic filopodia (precursors of the spines) start to elongate.

Apoptosis is necessary for proper CNS development—almost half of the immature neurons die in a process of programmed cell death before CNS is matured completely [30]. On the other hand, increased apoptosis occurs during neurodegenerative events [31]. It has been shown that GSK3β played a dual role in apoptosis promotion, depending on the organism's developmental stage. Inhibition of GSK3 (with lithium) promoted apoptosis in immature neurons, while in mature neurons, it supported cell survival [32]. In vivo, GSK3β can be activated in response to environmental conditions fluctuations, lack of trophic factors, cellular stress (oxidative, endoplasmic, DNA damage), which leads to increased apoptosis in mature cells [33][34][35]. GSK3β can induce apoptosis in two ways—by phosphorylation of microtubule-associated protein Tau, which leads to MT destabilization and cytoskeleton collapse [36], or by interaction with several transcription factors and proapoptotic proteins. Tau interacts with MTs maintaining its dynamics. The presence of hyperphosphorylated Tau protein is characteristic of neurodegenerative processes and causes the formation of Tau aggregates, which leads to MT disorganization that is correlated with apoptotic cell death induction [36].

3. GSK3–Cytoskeleton Interplay in Cell Motility and Migration of Cancer Cells

Cell motility is a random process of cell movement, while cell migration plays a role in organ and tissue formation, but it is also used by cancer cells to spread in a process known as metastasis (for review see [37]).

The role of GSK3 in cell migration and motility is still controversial, and although most studies have demonstrated that active GSK3 stimulated these processes [38][39][40][41][42], there are also reports indicating that active GSK3 inhibited cell movement [43][44][45].

Cell movement depends on the activity of two factors regulating antagonistic modes of cell migration, the mesenchymal (driven by Rac1-GTPase) and amoeboid (driven by RhoA-GTPase) mode. Rac1 downregulates RhoA expression via p190RhoGAP and PAK/GEF-H1 and, reciprocally, RhoA downregulates Rac1 via ROCK/FilGAP and ROCK/ARHGAP22. During the mesenchymal mode, the movement is driven by the formation of actin-rich protrusions called lamellipodia, and cells interact with the extracellular matrix (ECM) through focal adhesions (FAs). The amoeboid mode is driven by protrusions called blebs and it is FA independent. Cells undergoing amoeboid movement are characterized by rounded cell morphology than cells using the mesenchymal mechanism (for review see [46][47]).

Cancer cells exhibit various modes of migration. For example, U87MG glioblastoma cells are strictly mesenchymal mode cells, and Rac1 signaling inhibition blocks their movement [48]. In other mesenchymal mode cells, the HT1080 fibrosarcoma, suppression of Rac1 signaling does not inhibit the movement but leads to mesenchymal-to-amoeboid transition. Subsequent inhibition of RhoA signaling entirely blocks their motility. In turn, the amoeboid SW480 human colon adenocarcinoma cells adopt mesenchymal phenotype after RhoA or ROCK inhibition [48].

Although a direct effect of GSK3 on the mechanisms of cell migration has not been studied, there are numerous references documenting the effects of GSK3 on the activity of essential proteins involved in cell movement.

4. Conclusions

For many years, scientists and clinicians have focused their attention on the biochemical mechanisms of GSK3 and its involvement in various diseases. Additionally, for many years, this kinase has been indicated as a potential target of various therapies, and thus, efforts have been concentrated on exploring the possibility of using GSK3 inhibitors as potential drugs. Unfortunately, stunning success has not been frequently observed. In the way of achieving success stands probably the very reason why GSK3 is such an interesting object of research: multifunctionality of the kinase and complexity of functional cross-talks between GSK3 and its up- and downstream effectors. Global inhibition or overexpression of GSK3 in the cell rarely produces the desired results, often leading instead to serious side effects. Thus, although numerous GSK3 inhibitors have been evaluated, they relatively rarely reach Phase-2 clinical trials. The conclusions drawn in the experimental papers reviewed in our paper suggest that also in the case of the cytoskeleton-related cellular roles of GSK3, e.g., regulation of division, migratory potential, and the polarity of the cell, and maintaining transport of cargo along the cytoskeleton, a precise balance between activation and inhibition of the kinase and its proper spatiotemporal distribution is required. Thus, focusing on finding methods that enable only local alteration of activity/concentration of this protein, or targeting a specific downstream target or an upstream regulator of GSK3 might be a more promising approach than changing the total cellular activity/concentration of the kinase. Therefore, a comprehensive description of the cellular interaction networks of GSK3 might be crucial to identify novel protein targets with the highest therapeutic potential for the treatment of GSK3-related diseases.

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