A NEW INSIGHT OF GSK3B REGULATION: IMPLICATIONS IN CANCER THERAPY

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Abstract

Glycogen synthase kinase (GSK)-3 has emerged as one of the most attractive therapeutic targets for the treatment of multiple neurological diseases, including Alzheimer’s, stroke and bipolar disorders, as well as noninsulin-dependent diabetes mellitus and inflammation. Recent studies have revealed that GSK3β is a key regulator of numerous signaling pathways and is involved in a wide range of cellular processes, ranging from glycogen metabolism to cell cycle regulation, proliferation and tumorigenesis. The prominent role of GSK3β in the adenomatous polyposis coli (APC)–β-catenin destruction complex indicates that inhibition of GSK3β could possibly lead to tumor promotion through the activation of β-catenin. Inhibition of PI3K/AKT pathway activates GSK3β leading to G1 cell cycle arrest and apoptosis in human colon carcinoma HT29 cells. Also, GSK3β is a critical regulator of nuclear factor NF-κB nuclear activity, suggesting that inhibition of GSK3β could be effective in the treatment of a wide variety of tumors with constitutively active NF-κB. However, GSK3β also mediates drug sensitivity/resistance in cancer chemotherapy. Several other recent studies have shown the activity of GSK3β in cancer that provides new insight of the molecular mechanisms, through which it regulates tumor cell proliferation and survival of multiple human malignancies. This review addresses the molecular mechanism of its regulation through different signaling pathways.

Introduction

Glycogen synthase kinase 3β (GSK3β) is a serine/threonine kinase. Initially it was identified as a critical mediator in glycogen metabolism and insulin signaling. It is now known that GSK3β is a multifunctional kinase; it regulates more than 40 proteins depending on the cellular pathway and according to the need of cellular metabolism, including transcription factors, cell cycle/survival regulators and oncogenic/proto-oncogenic proteins (Doble et al., 2003; Jope et al., 2004). There are two mammalian GSK3 isoforms GSK3α and GSK3β encoded by distinct genes which share 85% identity
The two genes map to human chromosomes 19q13.2 (GSK3α) and 3q13.3 (GSK3β). Despite a high degree of similarity and sequence overlap, these isoforms are not functionally identical and redundant. The signaling pathway and protein function of GSK3β are extensively investigated (Plytes et al., 1992). Due to its diverse cellular functions, GSK3β acts as a key regulator of many pathways. Its deregulation has been implicated in the development of a number of human diseases such as diabetes, cardiovascular disease, some neurodegenerative diseases and bipolar disorder (Doble et al., 2003; Grimes et al., 2001; Beurel et al., 2006). However, the deregulation of GSK3β has also been implicated in tumorigenesis and cancer progression (Billadeau et al., 2007; Manoukian et al., 2002; Ougolkov et al., 2006; Seldin et al., 2005). Unlike most protein kinases, GSK3β is constitutively active in resting cells and undergoes a rapid and transient inhibition in response to a number of external signals (Doble et al., 2003; Grimes et al., 2001) and its activity is regulated by site-specific phosphorylation. The phosphorylation at tyrosine (Tyr-216) activated GSK3β and conversely, phosphorylation at serine (Ser-9) inhibits its activity. GSK3β is subjected to multiple regulatory mechanisms and phosphorylation at Ser-9 is probably the most important regulatory mechanism. Additionally, GSK3β regulates many proto-oncogenes or tumor suppressing transcription and translation factors. Tumor suppressor transcription factor p53 is a target of GSK3β and it regulates the level as well as intracellular localization of p53 (Beurel et al., 2006) and forms a complex with nuclear p53 to promote p53-induced apoptosis. GSK3β directly modulates the activity of transcription factors, activator protein 1 (AP-1) and nuclear factor-κB (NF-κB) (Grimes et al., 2001; Manoukian et al., 2002; Ougolkov et al., 2006). Both of these transcription factors play a critical role in neoplastic transformation and tumor development. However, the underlying mechanism(s) of GSK3β regulation in neoplastic transformation and tumor development are unclear. It remains controversial whether GSK3β is a “tumor suppressor” or an “oncogene”. In the following sections we will discuss our current understanding about the role of GSK3β in cancer progression and the molecular mechanism of its regulation with an implication to targeted therapy for the cancer treatment.

**PI3K/AKT and Wnt Signaling pathway mediated regulation of GSK3β in carcinogenesis**

The PI3K/AKT pathway is involved in GSK3β inactivation in many cell types (Ougolkov et al., 2006). Moreover, the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway is a crucial regulator of many normal cellular processes such as cell growth, proliferation, motility, survival and apoptosis. It is deregulated in a wide range of human cancers by gain or loss of function of several components of this pathway including PIK3CA, AKT and PTEN (Cully et al., 2006; Vogelstein et al., 2004; Vivanco et al., 2002). In response to various growth factors, PI3K phosphorylates and activates protein kinase B (PKB)/AKT. AKT1 is activated through phosphorylation at Ser-473 and Thr-308, which in turn phosphorylates GSK3β at serine residues in the N-terminus (Ser-9 and Ser-21) and inhibiting the GSK3β activity (Cros et al., 1995). Several studies have demonstrated the role of GSK3β in cell proliferation and apoptosis induction (Ougolkov et al., 2006).
In addition to PI3K/AKT signaling dependent regulation of GSK3β, it has also been seen that GSK3β is involved in the regulation of β-catenin signaling. It participates in the formation of a multi-component destruction complex that promotes the phosphorylation at regulatory domain of β-catenin and subsequent degradation of β-catenin suggesting an important role in regulating cell proliferation during progression in prostate cancer (Mulholland et al., 2006). Over activation of β-catenin signaling is involved in many forms of human cancer. This classical mode of GSK3β action should qualify it as a “tumor suppressor” since GSK3β is a critical, negative regulator both of PI3K and Wnt cell signaling (Ding et al., 2000). However, two recent studies have implicated that GSK3β may play a pro-tumor role in pancreatic and colorectal cancers (Ougolkov et al., 2005; Shakoori et al., 2005). The ovarian tumors often exhibit increased expression of GSK3β, indicating the critical role of GSK3β in ovarian cancer cells (Cao et al., 2006).

GSK3β has strikingly different behavior than other protein kinases and has a high basal activity within the cell, through insulin mediated pathway and Wnt stimulation lead to a decrease in its kinase activity. This allows unphosphorylated β-catenin to accumulate in the cytoplasm and nucleus. By binding to TCF family transcription factors, nuclear β-catenin regulates transcription of target genes such as c-myc and cyclin D1. Mutations that perturb the function of the Axin-APC complex, such as truncation of APC or deletion of the GSK3β interacting sites of β-catenin, are present in 90% of colon cancers (Polakis et al., 2000). Sequestration of GSK3β within the axin complex does not appear to be sufficient to prevent cross-talk with components of the insulin pathway. AKT/PKB phosphorylation, however, does not elicit the Wnt response (Ding et al., 2000) since the effects of insulin and Wnt are different, even in cells responsive to both signals. So how GSK3β is regulated and how is cross-talk between the Wnt and insulin pathways prevented?

**GSK3 β is involved in the regulation of cell cycle and apoptosis**

GSK3β regulates a variety of genes that participate in cell cycle regulation and apoptosis. Moreover, GSK3β is also phosphorylated at Ser-9 by activated AKT1, in response to various cellular growth factors observed in multiple cancers. The stability of cyclin D1 is regulated by GSK3β which phosphorylates cyclin D1 at Thr286, triggering the ubiquitination and proteolytic degradation of cyclin D1 by the ubiquitin-proteasome system (Matsushima et al., 1994). Cyclin D1 plays a critical role in G1 progression via phosphorylation of retinoblastoma (Rb), which regulates the transcription of the genes required for G1/S transition (Alao et al., 2006). It has also been found that an anticancer alkaloid (Tetrandrine) treated HT-29 a human cancer cells showed down-regulation of AKT and up-regulation of GSK3β, indicating that tetrandrine led to inhibition of AKT and activation of GSK3β and up-regulation of p27kip1. Also, increased phosphorylation of cyclin D1 (Thr286) catalyzed by GSK3β after tetrandrine treatment results in its degradation. Additionally, tetrandrine activates caspase 3, and PARP cleavage to its characteristic 85 kDa fragment, suggesting that tetrandrine induced apoptosis (Chen et al., 2008). This indicates that GSK3β activation via inhibiton of AKT was involved in tetrandrine-mediated of G1 arrest and apoptosis regulatory proteins.
GSK3β is a cytosolic protein which is translocated into the nucleus when activated and acts as transcription factors (Cohen et al., 2001; Harwood et al., 2001). In HT-29 a human cancer cells, the level of p27kip1 a cell cycle regulatory protein, was significantly increased after tetrandrine treatment in a concentration-dependent manner (Chen et al., 2008). A similar increase in p27kip1 protein was observed following treatment with PI3K/AKT inhibitor wortmannin and LY294002. Conversely, GSK3β inhibitors LiCl and SB216763 blocked up-regulation of p27kip1. These results suggest that GSK3β activation via AKT inhibition plays a crucial role in G1 arrest and apoptosis, which is consistent with previous studies that AKT inhibition and GSK3β activation promote G1 arrest and apoptosis in cancer cells (Arico et al., 2002; Krystal et al., 2002; Ha et al., 2007). However, a specific GSK3β inhibitor (LY2119301) promotes a genotoxic agent Adriamycin-induced apoptosis in human colorectal cancer cells (HCT116) in a p53-dependent manner (Tan et al., 2005).

The role of GSK3β in cancer initiation and progression

Since GSK3β negatively regulates many proto-oncoproteins and cell cycle regulators, one would predict that GSK3β may suppress carcinogenesis. Several lines of evidence support that GSK3β functions as a “tumor suppressor” and represses cellular neoplastic transformation and tumor development. In contrast to its tumor suppressive role, a few studies also suggest that GSK3β may promote carcinogenesis and cancer progression. GSK3β protein over expression has been found in human ovarian, colon and pancreatic carcinomas (Ougolkov et al., 2006). Higher levels of GSK3β are also observed in liver tumors than in normal liver tissues in a mouse model of hepatic carcinogenesis (Gotoh et al., 2003). Consistent with its high expression in ovarian tumors, GSK3β is reported to positively regulate the proliferation and survival of human ovarian cancer cells both in vitro and in vivo (Cao et al., 2006). GSK3β also inhibits β-catenin by sequestration and promotion of β-catenin degradation. In contrast, the GSK3β inactivation (pGSK-3β) through the Wnt pathway allows β-catenin to accumulate within the nucleus, thus up-regulating cyclin D1 as well as other genes including c-myc, c-Jun, and fos (Diehl et al., 1998). Cyclin D1 as a proto-oncogene can facilitate cell cycle progress and proliferation of thyrocytes but may also modulate several different transcription factors during neoplastic transformation (Diehl et al., 2002). However, the Cyclin D1 regulation in the cell cycle is mediated through GSK3β signaling protein via phosphatidylinositol-3-kinase/Akt (PI3K/Akt) and the Wnt canonical pathways (Jung at al., 2010; Diehl et al., 1998; Takahashi-Yanaga et al., 2008).

GSK3β is a direct regulator of AP-1. AP-1 is a hetero-dimeric transcription factor complex composed of a jun family member and a FOS family member that binds the TRE DNA sequence (50-TGAGTCA-30). It is involved in a variety of cellular processes, including growth, survival and tumorigenesis (Eferl et al., 2003). GSK3β induced c-Jun phosphorylation inhibits DNA binding activity and suppresses AP-1 activity (Grimes et al., 2001). AP-1 activation is required for the transformation of epidermal cells and skin carcinogenesis (Saeg et al., 1995; Yong et al., 1999). The GSK3β is also capable to induce the activity of nuclear factor kappa B (NF-κB), a key transcription factor for
pro-inflammatory immune responses (Cohen et al., 2001) and homozygous deletion of the GSK3β gene in mice is embryonically lethal due to extensive liver degeneration caused by a defect in NF-κB activity (Hoeflich et al., 2000). Thus, the activity of GSK3β is tightly controlled primarily by phosphorylation of regulatory serine residues (Ser9 in GSK3β) leading to its inhibition, but also by protein complex-formation and subcellular localization (Jope et al., 2004).

**GSK3β a target for cancer chemotherapy could open new avenue**

GSK3β regulates several cellular processes including cancer progression. However, GSK3β also regulates cellular sensitivity/resistance to cancer chemotherapy. Increased expression of pGSK3β (Ser9) is observed in cisplatin-resistant ovarian cancer cell line (CP70) compared to its cisplatin-sensitive counterpart A2780 cells (Cai et al., 2007). High pGSK3β (Ser9) levels in CP70 cells suggest that suppressed GSK3β activity may account for their resistance to cisplatin. Inhibition of GSK3β by treatment with lithium significantly reduces cisplatin-induced apoptosis and raises the IC50 of cisplatin for ovarian cancer cells. GSK3β reactivation by exogenous expression of S9A GSK3β mutant or treatment with LY294002 sensitizes hepatoma cells to etoposide and camptothecin induced apoptosis (Beurel et al., 2005). It has also shown that pharmacological or siRNA mediated inhibition of GSK3β reduce NF-κB mediated gene transcription and inhibit the growth of cancers that show high NF-κB activity including pancreatic cancer (Ougolgov et al., 2005; 2006; 2007), since aberrant NF-κB activation has been linked to drug resistance in pancreatic cancer. Furthermore, Rapamycin is known to activate GSK3β; it enhances a chemotherapy drug paclitaxel-induced apoptosis in GSK3 β wild-type, but not in GSK3β null breast cancer cells (Dong et al., 2005), indicating that GSK3β mediates rapamycin-induced chemosensitivity. A similar report indicates that GSK3β activation sensitizes human breast cancer cells to chemo-therapy drugs, 5-fluorouracil, cisplatin, taxol or prodigiosin-induced apoptosis (Ding et al., 2007; Soto-Cerrato et al., 2007). Since, The GSK3β regulated differential responses to chemotherapy among tumor cell types, it behaves either as tumor suppressor or “oncogene”. Further investigation is required to understand the mechanisms of this differential effect, and the potential for rational combination of GSK3β inhibitors with other targeted agents for the treatment of cancer. Moreover, Glycogen synthase kinase 3β (GSK3β) has become one of the most attractive therapeutic targets for the cancer treatment and could open a new avenue in cancer therapy as a potential agent.

**Conclusion**

GSK-3 is a constitutively active serine-threonine kinase that can phosphorylate and inactivate a broad range of substrates including glycogen synthase, cyclin D1, p27, c-myc, c-jun, and β-catenin. Although GSK3β is a distinct serine/threonine kinase present in the mammalian genome, this enzyme has attracted attention for its role in a diverse range of cellular processes and its regulation through several signaling pathways that are important in cancer and other human diseases. Recently, GSK3β has been viewed as a potent target in the treatment of several human cancers due to its involvement in tumor
development and chemo-resistance. The available evidence indicates that the direct pharmacological GSK3β inhibitors like LiCl and SB216763 and indirect PI3K/AKT inhibitor LY294002, wortmannin and siRNA mediated knockdown may provide an effective therapeutic avenue for the treatment of tumors. Although the mechanisms underlying the differential effects of GSK3β remains to be elucidated being a putative tumor-suppressor, before introducing any GSK3β based target therapy for the cancer.

REFERENCES


Figure 1: Interactions of glycogen synthase kinase-3β (GSK3β) with PI3K/AKT signaling and Wnt signaling pathway. Note the interaction of different protein complexes that interact with GSK3β to regulate and direct its actions. The sequential activation of phosphoinositide 3-kinase (PI3K), and Akt inactivates GSK3β by phosphorylating on Ser9. In the absence of the wnt ligand, GSK3β is bound to axin in a complex with β-catenin (β-cat), casein kinase I (CKI) and adenomatous polyposis coli protein (APC). CKI phosphorylates β-catenin to prime it for phosphorylation by GSK3β, resulting in proteosomal degradation of β-catenin. Stimulation of the two associated receptors, frizzled receptor (Fz1-R) and the low-density lipoprotein (LDL)-related protein 5/6 (LRP5/6) receptor by wnt results in the recruitment of FRAT (frequently arranged in T cell lymphomas) and disheveled (dvl) into the GSK3β complex. This prevents the phosphorylation of β-catenin by GSK3β, enabling β-catenin to accumulate and translocate to the nucleus where it is a co-transcriptional activator of T cell factor/lymphocyte-enhancer-binding factor (TCF/LEF), facilitating gene expression and promoting cell proliferation.