Journal of Scientific Research
Banaras Hindu University, Varanasi

Vol. 56, 2012 : 25-34
ISSN : 0447-9483

A NEW INSIGHT OF GSK3B REGULATION: IMPLICATIONS IN CANCER THERAPY

Shyam Babu Prasad and Gopeshwar Narayan
Department of Molecular & Human Genetics,
Banaras Hindu University, Varanasi – 221 005, India

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

(Doble *et al*., 2003). 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 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, triggring the ubiquitination and proteolytic degradation of cyclin D1 by the ubiquitin-proteasome system (Matsushime *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 $p27^{kip1}$. 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 $p27^{kip1}$ a cell cycle regulatory protein, was significantly increased after tetrandrine treatment in a concentration-dependent manner (Chen *et al*., 2008). A similar increase in $p27^{kip1}$ protein was observed following treatment with PI3K/AKT inhibitor wortamannin and LY294002. Conversely, GSK3β inhibitors LiCl and SB216763 blocked up-regulation of $p27^{kip1}$. 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 progression 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 (Saez *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, wortamannin 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

Alao, J. P., Stavropoulou, A. V., Lam, E.W.(2006): Coombes RC, Vigushin DM. Histone deacetylase inhibitor, trichostatin A induces ubiquitin-dependent cyclin D1 degradation in MCF-7breast cancer cells. *Mol Cancer*, 20: pp. 5-8.

Arico, S., Pattingre, S., Bauvy, C., Gane, P., Barbat, A., Codogno, P., Ogier-Denis, E. (2002): Celecoxib induces apoptosis by inhibiting 3-phosphoinositide-dependent protein kinase-1 activity in the human colon cancer HT-29 cell line. *J. Biol. Chem.,* 277: pp. 27613-21.

Beurel, E., Jope, R.S. (2006): The paradoxical pro- and anti-apoptotic actions of GSK3 in the intrinsic and extrinsic apoptosis signaling pathways. *Prog Neurobiol*, 79: pp. 173–189.

Beurel, E., Kornprobst, M., Blivet-Van Eggelpoel, M. J., Cadoret, A., Capeau, J., Desbois-Mouthon, C. (2005): GSK-3beta reactivation with LY294002 sensitizes hepatoma cells to chemotherapy-induced apoptosis. *Int. J. Oncol*., 27: pp. 215–222.

Billadeau, D. D. (2007): Primers on molecular pathways. The glycogen synthase kinase 3beta. *Pancreatology*, 7: pp. 398–402.

Cai, G., Wang, J., Xin, X., Ke, Z., Luo, J. (2007): Phosphorylation of glycogen synthase kinase-3 beta at serine 9 confers cisplatin resistance in ovarian cancer cells. *Int. J. Oncol*., 31: pp. 657–662.

Cao, Q., Lu, X., Feng, Y. J. (2006): Glycogen synthase kinase-3beta positively regulates the proliferation of human ovarian cancer cells. *Cell Res*, 16: pp. 671–677.

Chen, X. L., Ren, K. H., He, H. W., Shao, R. G. (2008): Involvement of PI3K/AKT/GSK3β pathway in tetrandrine-induced G1 arrest and apoptosis. *Cancer Biology & Therapy*; 7(7): pp. 1073-1078.

Cohen, P., Frame, S. (2001): The renaissance of GSK3. *Nat. Rev. Mol. Cell Biol.*, 2:pp. 769-76.

Cros, D. A., Alessi, D. R., Cohen, P., Andjelkovich, M., Hemmings, B. A. (1995): Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase *B. Nature,* 378: pp. 785-9.

Cully, M., You, H., Levine, A. J., Mak, T. W. (2006): Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. *Nat. Rev. Cancer*, 6: pp. 184–92.

Diehl, J. A., Cheng, M., Roussel, M. F., Sherr C. J. (1998): Glycogen synthase kinase- 3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes Dev*, 12: pp. 499-511.

Diehl, J. A. (2002): Cycling to cancer with cyclin D1. *Cancer Biol. Ther*., 1: pp. 226-31.

Ding, Q., He, X., Hsu, J. M., Xia, W., Chen, C. T., Li, L. Y., Lee, D. F., Liu, J. C., Zhong, Q, Wang, X., Hung, M. C. (2007): Degradation of Mcl-1 by beta-TrCP mediates glycogen synthase kinase 3-induced tumor suppression and chemosensitization. *Mol. Cell Biol*., 27: pp. 4006–4017.

Ding, V. W., Chen, R. H., McCormick, F. (2000): Differential regulation of glycogen synthase kinase 3beta by insulin and Wnt signaling. *J. Biol. Chem*., 275: pp. 32475–32481.

Doble, B. W., Woodgett, JR. (2003): GSK-3: tricks of the trade for a multi-tasking kinase. *J. Cell Sci.*, 116: pp 1175–86.

Dong, J., Peng, J., Zhang, H., Mondesire, W. H., Jian, W., Mills, G. B., Hung, M. C. (2005): Meric-Bernstam F. Role of glycogen synthase kinase 3beta in rapamycin-mediated cell cycle regulation and chemosensitivity. *Cancer Res.,* 65: pp. 1961–1972.

Eferl, R., Wagner, E. F. (2003): AP-1: a double-edged sword in tumorigenesis. *Nat. Rev. Cancer* 3:pp. 859–868.

Gotoh, J., Obata, M., Yoshie, M., Kasai, S., Ogawa, K. (2003): Cyclin D1 over-expression correlates with beta-catenin activation, but not with H-ras mutations, and phosphorylation of Akt, GSK3 beta and ERK1/2 in mouse hepatic carcinogenesis. *Carcinogenesis*, 24: pp. 435–442.

Grimes, C. A., Jope, R. S. (2001): The multifaceted roles of glycogen synthase kinase 3beta in cellular signaling. *Prog. Neurobiol*, 65: pp. 391–426.

Ha S. D., Ng D, Pelech, S. L., Kim, S. O. (2007): Critical role of the phosphatidylinositol 3-kinase/Akt/ glycogen synthase kinase-3 signaling pathway in recovery from anthrax lethal toxin-induced cell cycle arrest and MEK cleavage in macrophages. *J. Biol. Chem*., 282: pp. 36230-9.

Harwood, A. J. (2001): Regulation of GSK-3: a cellular multiprocessor. *Cell*, 105: pp. 821-4.

Hoeflich, K. P., Luo, J., Rubie, E. A., Tsao, M. S., Jin, O., Woodgett, JR. (2000): Requirement for glycogen synthase kinase-3beta in cell survival and NF-kappaB activation. *Nature,* 406: pp. 86–90.

Jope, R. S., Johnson, G. V. (2004): The glamour and gloom of glycogen synthase kinase-3. Trends *Biochem. Sci*., 29: pp. 95–102.

Jung, C. K., Kang, Y. G., Bae, J. S., Lim, D. J., Choi, Y. J., Lee, K. Y. (2010): Unique patterns of tumor growth related with the risk of lymph node metastasis in papillary thyroid carcinoma. *Mod. Pathol,* 23: pp. 1201-8.

Krystal, G. W., Sulanke, G., Litz, J. (2002): Inhibition of phosphatidylinositol 3-kinase-Akt signaling blocks growth, promotes apoptosis and enhances sensitivity of small cell lung cancer cells to chemotherapy. *Mol. Cancer Ther.,* 1: pp. 913-22.

Lin, C. F., Chen, C., Chiang, C. W., Jan, M. S., Huang, W. C., Lin, Y. S. (2007): GSK-3beta acts downstream of PP2A and the PI3-kinase-Akt pathway, and upstream of caspase-2 in ceramide-induced mitochondrial apoptosis. *J. Cell Sci*., 20: pp. 2935-43.

Manoukian, A. S., Woodgett, JR. (2002): Role of glycogen synthase kinase-3 in cancer: regulation by Wnts and other signaling pathways. *Adv. Cancer Res*, 84: 203–229.

Matsushime, H., Quelle, D. E., Shurtleff, S. A., Shibuya, M., Sherr, C. J., Kato, J. Y. (1994): D-type cyclin-dependent kinase activity in mammalian cells. *Mol. Cell Biol*., 14: pp. 2066-76.

Mulholland, D. J., Dedhar, S., Wu, H. and Nelson, C. C. (2006): PTEN and GSK3β: key regulators of progression to androgen-independent prostate cancer. *Oncogene*, 25: pp. 329–337.

Ougolkov, A. V., Billadeau, D. D. (2006):Targeting GSK-3: a promising approach for cancer therapy? *Future Oncol*, 2: pp. 91–100.

Ougolkov, A. V., Bone, N. D., Fernandez-Zapico, M. E., Kay, N. E., Billadeau, D. D. (2007): Inhibition of glycogen synthase kinase-3 activity leads to epigenetic silencing of nuclear factor kappaB target genes and induction of apoptosis in chronic lymphocytic leukemia B cells. *Blood*, 110: pp. 735-742.

Ougolkov, A. V., Fernandez-Zapico, M. E., Bilim, V. N., Smyrk, T. C., Chari, S. T., Billadeau, D. (2006): Aberrant nuclear accumulation of glycogen syn-thase kinase-3beta in human pancreatic cancer: association with kinase activity and tumor dedifferentiation. *Clin Cancer Res*, 12: pp. 5074-5081.

Ougolkov, A. V., Fernandez-Zapico, M. E., Savoy, D. N., Urrutia, R. A., Billadeau, D. D. (2005): Glycogen synthase kinase-3β participates in nuclear factor κB-mediated gene transcription and cell survival in pancreatic cancer cells. *Cancer Res*, 65: pp. 2076-2081.

Ougolkov, A. V., Fernandez-Zapico, M. E., Savoy, D. N., Urrutia, R. A., Billadeau, D. D. (2005): Glycogen synthase kinase-3beta participates in nuclear factor kappaB-mediated gene transcription and cell survival in pancreatic cancer cells. *Cancer Res*., 65: pp. 2076-2081.

Plytes, S. E., Hughes, K., Nikolakaki, E., Pulverer, B. J., Woodgett, JR. (1992): Glycogen synthase kinase-3: functions in oncogenesis and development. *Biochim Biophys Acta,* 1114: pp. 147–162.

Polakis, P. (2000): Wnt signaling and cancer. *Genes Dev.,* 14: pp. 1837–1851.

Saez, E., Rutberg, S. E., Mueller, E., Oppenheim, H., Smoluk, J., Yuspa, S. H., Spiegelman, B. M. (1995): c-fos is required for malignant progression of skin tumors. *Cell*, 82: pp 721–732.

Seldin, D. C., Landesman-Bollag E, Farago, M., Currier, N., Lou, D., Dominguez, I. (2005): CK2 as a positive regulator of Wnt signalling and tumourigenesis. *Mol. Cell Biochem*., 274: pp. 63–67.

Shakoori, A., Ougolkov, A., Yu, ZW., Zhang, B., Modarressi, M. H., Billadeau, D. D., Mai M., Takahashi, Y., Minamoto, T. (2005): Deregulated GSK3β activity in colorectal cancer: its association with tumor cell survival and proliferation. *Biochem Biophys Res Commun*, 334: pp. 1365-1373.

Soto-Cerrato, V., Viñals, F., Lambert, JR., Kelly, JA., Pérez-Tomás, R. (2007): Prodigiosin induces the proapoptotic gene NAG-1 via glycogen synthase kinase-3beta activity in human breast cancer cells. *Mol. Cancer Ther*, 6: 362–369.

Takahashi-Yanaga, F., Sasaguri, T. (2008): GSK-3beta regulates cyclin D1 expression: a new target for chemotherapy. *Cell Signal,* 20: pp. 581-9.

Tan, J., Zhuang, L., Leong, H. S., Iyer, N. G., Liu, E. T., Yu, Q. (2005): Pharmacologic modulation of glycogen synthase kinase-3beta promotes p53-dependent apoptosis through a direct Bax mediated mitochondrial pathway in colorectal cancer cells. *Cancer Res*, 65: pp. 9012–9020.

Vivanco, I., Sawyers, C. L. (2002): The phosphatidylinositol 3-kinase AKT pathway in human cancer. *Nat. Rev. Cancer*, 2: pp. 489–501.

Vogelstein, B., Kinzler, K. W. (2004): Cancer genes and the pathways they control. *Nat. Med*., 10: pp. 789–99.

Young, M. R., Li, J. J., Rincon, M., Flavell. R. A., Sathyanarayana, B. K., Hunziker, R., Colburn, N. (1999): Transgenic mice demonstrate AP-1 (activator protein-1) transactivation is required for tumor promotion. *Proc. Natl. Acad. Sci.,* USA; 96: pp. 9827–9832.

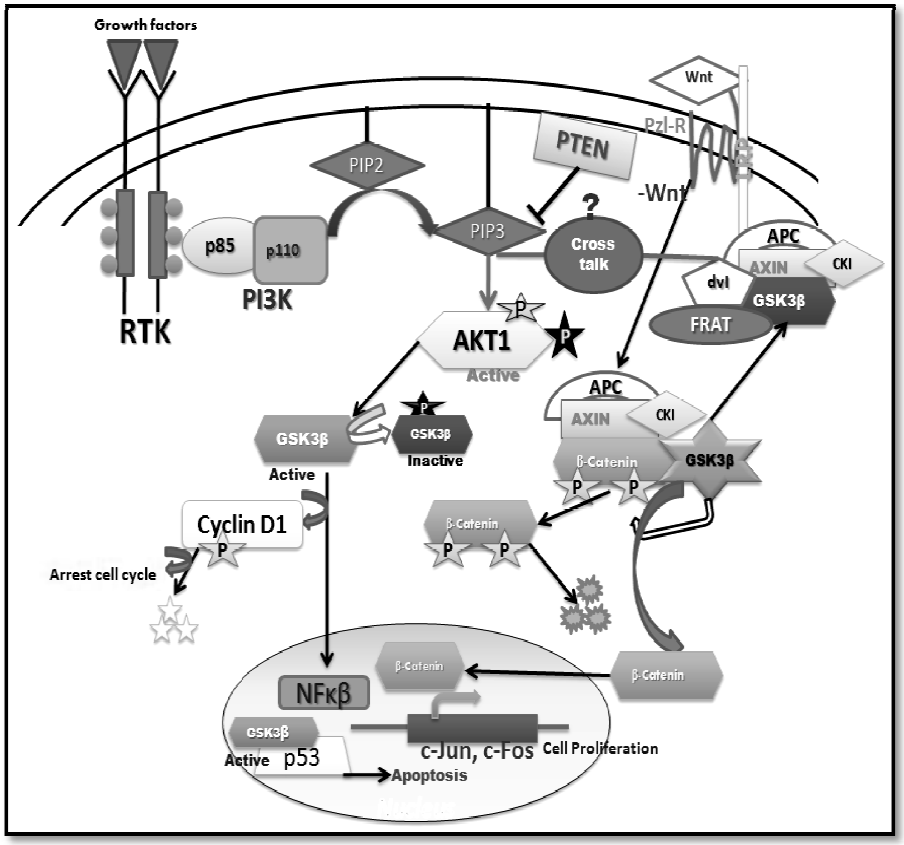

Figure 1: Interactions of glycogen synthase kinase-3β (GSK3β) with PI3K/AKT signaling and Wnt signaling pathway. Note the interaction of different protein complexes that interact with GSK3β to regulate and direct its actions. The sequential activation of phosphoinositide 3-kinase (PI3K), and Akt inactivates 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 (Fzl-R) and the low-density lipoprotein (LDL)-related protein 5/6 (LRP5/6) receptor by wnt results in the recruitment of FRAT (frequently arranged inT 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.