An emerging strategy for cancer treatment targeting aberrant glycogen synthase kinase 3 β

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An Emerging Strategy for Cancer Treatment Targeting Aberrant Glycogen Synthase Kinase 3β

Short title: Targeting GSK3β for Cancer Treatment

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Abstract

Improvement in the outcome of cancer patients who are refractory to currently available treatments relies on the development of target-directed therapies. One group of molecular targets with potential clinical relevance is a set of protein tyrosine kinases encoded mostly by proto-oncogenes and that are frequently deregulated in cancer. Glycogen synthase kinase 3β (GSK3β), a serine/threonine protein kinase, has emerged as a therapeutic target for common chronic diseases including type 2 diabetes mellitus, neurodegenerative disorders, inflammation and osteoporosis. This is based on its currently known functions and primary pathologic causalities. GSK3β has well characterized roles in the regulation of gene transcription and in oncogenic signaling. We have shown that deregulated GSK3β promotes gastrointestinal, pancreatic and liver cancers and glioblastomas. Furthermore, we have demonstrated that inhibition of GSK3β attenuates cell survival and proliferation, induces cell senescence and apoptosis and sensitizes tumor cells to chemotherapeutic agents and ionizing radiation. This has led us to propose GSK3β as a potential therapeutic target in cancer. The anti-tumor effects of GSK3β inhibition are mediated by changes in the expression and phosphorylation of molecules critical to the regulation of cell cycling, proliferation and apoptosis and underlie the pathological role for GSK3β in cancer. Investigation of the mechanisms responsible for deregulation of GSK3β and the consequent downstream pathologic effects in cancer cells has shed light on the molecular pathways leading to tumorigenesis. This will allow exploration of novel therapeutic strategies for cancer that target aberrant GSK3β.

Key words: glycogen synthase kinase 3β, molecular target, cancer

GSK3β AND COMMON DISEASES

Glycogen synthase kinase 3β (GSK3β) was first identified as a serine/threonine protein kinase that regulates glucose/glycogen metabolism under the control of insulin signaling. Unlike most protein kinases, GSK3β is active in normal cells and this activity is controlled by its subcellular localization, differential phosphorylation and different binding partners. GSK3β is inactive when its serine 9 (S9) residue is phosphorylated by the actions of PKA (protein kinase A), Akt/PKB (protein kinase B) and/or PKC (protein kinase C). It is active when its tyrosine 216 (Y216) residue is phosphorylated either through autophosphorylation or through the action of other undetermined kinase(s) [1-3]. This suggests that GSK3β activity is regulated by the balance between phosphorylation levels at its S9 and Y216 residues. An alternate, phosphorylation-independent mechanism for regulating GSK3β kinase activity has also been reported recently [4]. GSK3β not only regulates its primary substrate, glycogen synthase (GS), but also influences other fundamental cellular pathways depending on the substrates that it phosphorylates and the partners that it binds and interacts with [1-3].

Accumulating evidence has implicated GSK3β in the development of adult onset, chronic diseases including type 2 (non-insulin-dependent) diabetes mellitus (NIDDM) [5-7] and Alzheimer's disease [8, 9]. Recognition that GSK3β promotes inflammation indicates that it has pathological roles in a wide range of prevalent diseases including NIDDM and neuropsychiatric disorders that involve an inflammatory component [10]. Accordingly, GSK3β has emerged as a potential target for the development of drugs against these prevalent chronic diseases because of its causative associations with glucose intolerance, neuronal cell death and inflammation [11-13]. Another line of investigation has shown that signaling via the Wnt/β-catenin pathway facilitates osteogenesis [reviewed in 14-16]. This suggests that GSK3β could also be a therapeutic target in osteoporotic bone disorders, since it is a well established negative regulator of the Wnt/β-catenin pathway in normal cells (described below). In this context, an orally bioavailable GSK3α/β dual inhibitor has been generated as a new drug for the treatment of osteoporosis [17].

GSK3β IN CANCER

Under physiological conditions, GSK3β phosphorylates and thereby triggers the degradation of several transcription factors (eg. c-Jun, c-Myc), cell cycle regulators (eg, cyclin D1) and proto-oncoproteins such as β-catenin [18, 19]. It is therefore believed to inhibit tumor development by interfering with oncogenic signaling pathways (eg. Wnt, Hedgehog) [20]. A recent study showing that GSK3β phosphorylates and stabilizes a cell cycle regulator $p27^{Kip1}$ also suggests a tumor suppressor function of GSK3β [21]. Other lines of study indicate that GSK3β plays crucial roles in nuclear factor-κB (NF-κB)-mediated cell survival [22, 23] and Notch stability and signaling [24]. It is clear that the tumor microenvironment, which is largely orchestrated by inflammatory cells, plays an important part in cellular neoplastic transformation by fostering tumor cell proliferation, survival and invasion [25, 26]. As described above, the close association of GSK3β with inflammation [10] suggests a putative pathological role for GSK3β in cancers that involve inflammation. In addition, several studies have demonstrated that GSK3β destabilizes the tumor suppressor proteins p53 [27, 28] and PTEN (phosphatase and tensin homologue deleted on chromosome 10) [29], indicating that it may promote cancer. These conflicting notions of the biological functions of GSK3β have prompted investigations into the pathologic roles of GSK3β in cancer, which is characterized by the irreversible deregulation of cell survival, proliferation and differentiation [30].

GSK3β suppresses tumor growth

In line with the hypothesis that GSK3β acts as a tumor suppressor [20, 21], a number of studies on breast, lung and non-melanoma skin cancers have shown that GSK3β is inactivated in cancer tissues and that its activation can induce the apoptosis of cancer cells [31-38, reviewed in 39, 40]. It has also been reported that inhibition of GSK3β promotes epithelial-mesenchymal transition (EMT), a morphological change hypothesized to associate with the invasion and metastasis of tumor cells [41, 42, reviewed in 43-45]. GSK3β can also render cancer cells resistant to chemotherapeutic agents [46-50, reviewed in 40]. However,

most studies have not evaluated for differences in the biological properties of GSK3β between tumor cells and their normal counterparts, nor the consequences of GSK3β inhibition for tumor cell survival, proliferation and migration.

Deregulated GSK3β promotes cancer

We have previously described aberration of the ubiquitin system and its involvement in oncogenic Wnt signaling in colorectal cancer [51-56]. Since GSK3β interrupts the canonical Wnt pathway by targeting β-catenin for phosphorylation and subsequent ubiquitin-mediated degradation [18-20], we directed our research focus towards the study of a putative role for GSK3β in cancer. We found increased expression and activity of GSK3β and deregulated activity due to imbalance in the differential phosphorylation of S9 and Y216 residues in colon cancer cell lines and primary colorectal cancers when compared with their normal counterparts. These properties of GSK3β were unrelated to the activation of β-catenin or Akt/PKB [57]. The latter is an upstream kinase that phosphorylates the GSK3β S9 residue [1-3]. Using a non-radioisotopic *in vitro* kinase assay (NRIKA) [58], we detected increased activity of GSK3β not only in colon cancer cells but also in stomach, pancreatic and liver cancer cells and glioblastomas compared to the corresponding normal tissues in which GSK3β activity appears to be regulated by differential phosphorylation [59-60]. These observations suggest that GSK3β may promote cancer, in contrast to its hypothetical anticancer functions.

A putative pathological role for GSK3β in cancer was demonstrated by our observations that its inhibition by pharmacological (small-molecule) inhibitors and RNA interference (RNAi) reduced the survival of various cancer cell types and predisposed them to undergo apoptosis *in vitro* [57-60] and in tumor xenografts [60-62]. This led us to propose that aberrant GSK3β is a potential therapeutic target for cancer treatment and enabled us to apply for domestic and international patents entitled “Suppression of cancer and method for evaluating anticancer agent based on the effect of inhibiting GSK3β” [63]. At the same time or shortly after our studies on the anti-tumor effects of GSK3β inhibition [57-63], similar

observations were reported in prostatic [64, 65], colon [66-68], pancreatic [69, 70], ovarian [71], esophageal [72] and medullary thyroid [73, 74] cancers, as well as melanoma [75], hematologic malignancies [76-81], malignant gliomas [82, 83], pheochromocytoma and paraganglioma [84] (Table 1). As mentioned earlier, although the exact role of GSK3β in cancer is still being debated, the overall conclusion from these studies is that GSK3β is likely to be a promising therapeutic target in a range of cancer types.

The hypothesis of oncogene addiction has been proposed as a rationale for molecular targeting in cancer treatment. It refers to the observation that a cancer cell, despite its plethora of genetic alterations, seemingly exhibits dependence on a single oncoprotein or oncogenic pathway for its sustained survival and/or proliferation [85, 86]. This unique state of dependence in cancer cells is highlighted by the fact that inactivation of the normal counterpart of such oncogene products in normal cells is tolerated without obvious consequence. A profound implication of this hypothesis is that acute interruption of crucial oncogenic pathways upon which cancer cells are dependent should have major detrimental effects on the cancer cells while sparing normal cells that are not similarly addicted to these pathways [87]. In our series of studies, inhibition of GSK3β had little effect on cell survival, growth, apoptosis or senescence in non-neoplastic cells (eg. HEK293) [57-63]. This is supported by reports showing that GSK3β inhibition does not influence the survival or growth of human mammary epithelial cells, embryonic lung fibroblasts (WI38) and mouse embryonic fibroblasts (NIH-3T3) [69, 73]. Consistent with the physiological roles of GSK3β in Wnt and Hedgehog signaling, GSK3β inhibition by pharmacological means can promote embryonic stem cell pluripotency [88] and hematopoietic stem cell reconstitution [89]. With respect to the oncogene addiction hypothesis [85-87], the selective therapeutic effect of GSK3β inhibition against cancer can be explained by our observations on the distinct properties of GSK3β in cancer cells [57-63]. In contrast to the effect seen against malignant gliomas [59, 82, 83], an additional therapeutic benefit of GSK3β inhibition was highlighted by a recent study showing that it protects hippocampal neurons from radiation-induced damage, thus preventing neurocognitive dysfunction resulting from cranial irradiation [90].

GSK3β INHIBITORS ACT AGAINST CANCER

Different types or classes of GSK3β inhibitors exist based on their structure and mechanism of action. Synthetic small-molecule inhibitors have frequently been used for investigations into the normal functions and pathological properties of GSK3β, but not for the treatment of diseases in clinical settings. Unlike protein tyrosine kinases, there are no published studies that have investigated the inhibitory effects of specific antibodies on GSK3β function in normal or cancer cells.

Chemical compounds (classical inhibitors)

The best known non-competitive inhibitor of GSK3β *in vitro* and *in vivo* is lithium [91, 92]. Lithium has been used for more than 50 years as a mood stabilizer and still constitutes the primary treatment for bipolar disorder [reviewed in 11-13]. Although the mechanism of action is unclear [93], lithium ions inhibit GSK3β activity by competing with magnesium ions (Mg^{2+}) and/or increasing phosphorylation of the S9 residue [94]. Some studies have shown that increased levels of GSK3β S9 phosphorylation were associated with the therapeutic effects of lithium in cancer cells [68, 72-74, 83, 84].

Valproate (2-propylpentanoic acid), a short-chain fatty acid, is a widely prescribed drug for the treatment of epilepsy and bipolar disorder. Several studies [95-98] have shown that valproate inhibits histone deacetylase (HDAC), a nuclear enzyme that plays a crucial role in chromatin remodeling and that has been implicated in cancer development and progression. Inhibition of HDAC leads to changes in the aberrant chromatin structure of cancer cells [99, 100] and valproate has therefore emerged as a potent anti-cancer agent [101-103]. Clinical trials for the treatment of cancer using valproate alone or in combination are ongoing [104, 105]. Recently, two phase I clinical trials were reported using valproate with the methyltransferase inhibitor 5-azacytidine or with the topoisomerase II inhibitor epirubicin [106, 107]. These revealed the pharmacokinetic properties and toxicity profiles of valproate in cancer patients, as well as reporting rates of stable disease as 39% and partial response as 22%. In addition to inhibiting HDAC, valproate was also found to directly inhibit GSK3β

[108, 109]. Many of the cancer types included in the above mentioned clinical trials with valproate [104-107] are also those reported to be responsive to treatment with GSK3β inhibitors (Table 1). Therefore, the dual inhibition of HDAC and GSK3β by valproate may provide a basis for its anti-cancer activity.

Pharmacological (small-molecule) inhibitors

More than 30 small-molecule (molecular weight < 600) inhibitors of GSK3β have been described to date. Despite their wide chemical diversity, most pharmacological inhibitors share the common properties of: (a) they are rather flat, hydrophobic heterocycles; (b) most, but not all, act by competing with ATP in the ATP-binding site of the kinase; (c) similar to cyclin-dependent kinase (CDK) inhibitors, they bind with the kinase through hydrophobic interactions and 2-3 hydrogen bonds [reviewed in 12]. The most frequently used compounds for inhibiting GSK3β in cancer cells include SB216763, SB415286, AR-A014418 and BIO (Table 1, Figure 1). Of note, it has been reported that AR-A014418 does not significantly inhibit 26 closely related protein kinases and is therefore considered highly specific for GSK3β [110]. These inhibitors efficiently suppress the proliferation of tumor cells and induce their apoptosis *in vitro* and in tumor xenografts (Table 1) within the reported pharmacological doses [12, 110].

Nucleic acid inhibitors (siRNA, shRNA)

Many of the studies shown in Table 1 used RNAi with small interfering (si) or short hairpin (sh) RNA specific to GSK3β in order to evaluate the effect of GSK3β inhibition on cancer cells and to investigate the molecular mechanism. Similar to the effects of pharmacological inhibitors, the consequences of selective knockdown of GSK3β in cancer cells included decreases in cell survival and proliferation and the induction of apoptosis. This indicates that both GSK3β expression and activity are necessary for tumor cell survival and proliferation in the cancer types shown in Table 1.

While many studies have suggested that GSK3β is a promising target for drug

development [11-13], none of the available small-molecule and nucleic acid inhibitors has yet found clinical use for the treatment of diseases such as diabetes mellitus, Alzheimer's disease, inflammation or cancer. This is because of a suspected increased risk for tumorigenesis through activation of the Wnt or Hedgehog signaling pathways following GSK3β inhibition, as well as the multiple functions of GSK3β in normal cellular metabolism and signaling [1-3, 11, 18-20, 39, 40]. Previous studies have suggested that GSK3β may have tumor suppressor-like functions based on its roles in Wnt/β-catenin signaling, the expression of cyclin D1, c-Myc and cyclooxygenase-2 (COX-2), the activity of extracellular signal–regulated kinase 1/2 (ERK1/2) and EMT [reviewed in 20, 39, 40]. However, none of these studies has conclusively shown neoplastic transformation or tumor development following inhibition of GSK3β. The differential effects of GSK3β inhibition on neoplastic and non-neoplastic cells [57-63, 69, 73] may occur because of differences in the biological properties and functions of the kinase between these cell types. This would support the potential clinical application of GSK3β inhibitors for the treatment of cancer types other than those of skin, breast and lung. Even if the continuous inhibition of GSK3β was to initiate the development of a second cancer in a patient with refractory primary cancer, this would be expected to develop clinically only after a considerable lag period. Therefore, any secondary tumorigenic potential associated with inhibition of GSK3β may not be of clinical concern unless the primary cancer is cured by treatment with the GSK3β inhibitors.

Drugs in clinical use

Recent structure-based *in silico* screening has demonstrated that a number of drugs currently in clinical use, other than lithium and valproate, may inhibit GSK3β activity. These include cimetidine, hydroxychloroquine (an anti-malarial and anti-lupus erythematodes agent), gemifloxacin (a new quinolon antibiotic) and olanzapine (an atypical antipsychotic agent) (Figure 1), all of which have been found to increase the level of glycogen in the rodent liver [111, 112]. Based on their effect against GSK3β, these agents are candidates for potent anti-cancer treatments. It has been reported that cimetidine, a histamine H2 receptor

antagonist, has anti-tumor activity against colon, stomach and kidney cancers and melanomas. This activity appears to involve a number of different mechanisms including inhibition of angiogenesis, antagonism of the tumor promoting effect of histamine via activation of H2 receptor, and enhancement of the host immune response to tumor cells [reviewed in 113]. Inhibition of GSK3β may be another mechanism by which cimetidine exerts anti-cancer activity.

Natural compounds

Libraries of natural compounds are sources from which new bioactive molecules are identified. Recently, two groups of natural compounds have been shown to inhibit GSK3β activity. One group includes tautomycin and tautomycetin, two antifungal antibiotics originally isolated from *Streptomyces spiroverticillatus* and *Streptomyces griseochromogens*, respectively [114, 115]. Tautomycetin was previously reported to inhibit the proliferation of colorectal cancer cells through $p21^{Cip/Waf1}$ induction via the ERK pathway [116]. Both tautomycin and tautomycetin suppress the growth of medullary thyroid cancer cells via inhibition of GSK3β [74]. The other group includes a number of compounds derived from benzofuran-3-yl-(indol-3-yl)maleimides, some of which have been identified as GSK3β inhibitors that suppress the proliferation and survival of pancreatic cancer cells [117].

MOLECULAR BASIS FOR TARGETING GSK3β IN CANCER

There is considerable interest in the molecular mechanism by which GSK3β exerts a putative pathological role in the promotion of tumor cell survival and proliferation (Tables 1 and 2). Following the discovery by our group of a novel pathological role for GSK3β in colorectal cancer [57, 58, 61, 63], several groups have investigated the molecular mechanisms by which GSK3β may promote cancer. These include various roles for GSK3β in tumor cell resistance to apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), p53 and c-Myc [27, 28, 64, 68], NF-κB-mediated gene transcription [69, 77, 82], induction of cyclin D1 expression [71], and the phosphorylation and destabilization of PTEN

[29]. GSK3β has also been reported to inhibit colonocyte differentiation by destabilizing the transcription factor Hath1 [118] and to facilitate cell migration by binding to h-prune and modulating focal adhesions and Rac-1 activity [119-122]. These effects were observed in cells of non-neoplastic origin (Table 2) and should therefore also be investigated in cancer cells. In all glioblastoma cell lines analyzed to date, inhibition of GSK3β increases the expression of p53 and p21 in cells with wild-type p53 and decreases Rb phosphorylation and CDK6 expression [59]. By analyzing the molecular changes in colon cancer cells transfected with GSK3β-specific siRNA, we recently observed a decrease in the level of human telomerase reverse transcriptase (hTERT) mRNA following GSK3β depletion. Inhibition of GSK3β attenuated telomerase activity and increased β-galactosidase-positive colon cancer cells. These effects were associated with increased expression of p53, p21 and JNK1 (c-Jun N-terminal kinase 1) and decreases in CDK6 expression and Rb phosphorylation [60]. Our findings were consistent with known relationships between such proteins and cell senescence [123] and with GSK3β activity [27, 28, 60, 66, 124, 125]. The putative role of GSK3β for maintaining the survival of cancer cells may therefore be partly due to effects on hTERT expression, telomerase activity and cellular senescence. The latter effect is consistent with a recent report that enhanced glycogenesis is directly linked to cellular senescence via the modulation of GSK3α/β and glycogen synthase [126].

CLINICAL IMPLICATIONS

The use of GSK3β inhibitors to treat chronic diseases requires a strong awareness of safety issues. Because GSK3β is a multi-task kinase, its systemic inhibition could lead to unexpected side effects arising from the disruption of normal metabolism and/or cellular signaling. However, in our preclinical studies and those of others, no detrimental effects were observed in rodents treated with GSK3β inhibitors [60-62]. Post-translational modification of GSK3β by phosphorylation is thought to underlie the mechanism by which normal cells are protected from undesirable effects. Because of the role of GSK3β in regulating various proto-oncoproteins, there are concerns that long-term inhibition of GSK3β could increase the

risk of cancer development [11, 20, 39, 40]. However, it has been reported that long-term administration of lithium, the classical GSK3β inhibitor, did not increase mortality from cancer but was instead associated with a reduction in the overall mortality of patients with bipolar disorder [127]. Lithium is not thought to be mutagenic or carcinogenic [128] and treatment with this compound did not significantly increase the incidence of intestinal tumors in genetically predisposed *APC* mutant mice [129]. This is consistent with the absence of primary tumor development in rodents from our preclinical studies [60-62]. Inhibition of GSK3β is not sufficient to stabilize β-catenin in normal cells and this seems to occur only when one or more transforming events such as APC protein truncation has already taken place [130]. Furthermore, in normal cells the critical function of GSK3β in mediating Wnt/β-catenin signaling is performed by cell membrane-associated GSK3β. This antagonizes the phosphorylation of β-catenin by cytoplasmic GSK3β and thus its degradation [131]. Therefore, the available evidence suggests that any increased risk of cancer associated with long-term GSK3β inhibition can be avoided by generating new compounds that spatially and temporally regulate the expression and activity of GSK3β.

DISCLOSUR OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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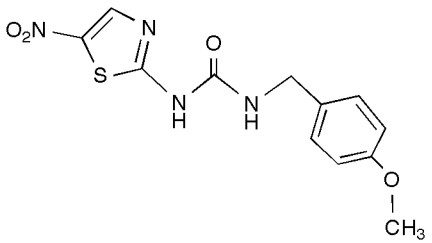
FIGURE LEGEND

Figure 1. Chemical structures of small-molecule inhibitors for GSK3β and of drugs in clinical use that have been found to inhibit GSK3β activity.

Figure 1



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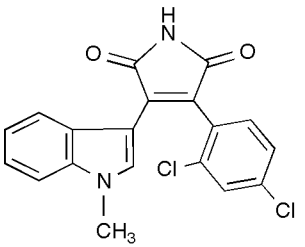

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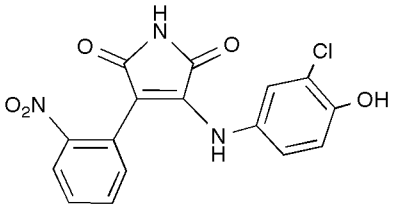

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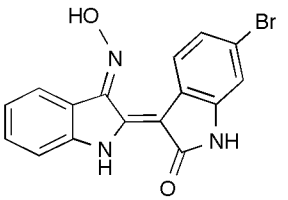

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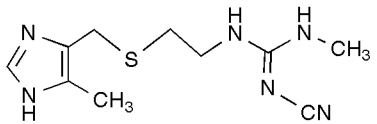

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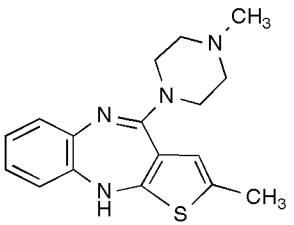

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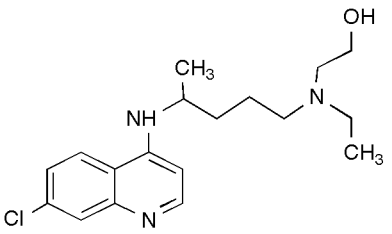
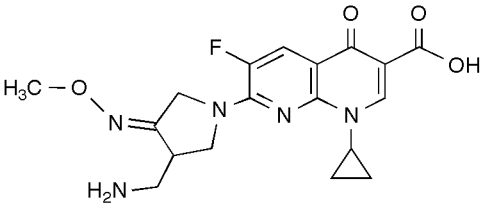


hydroxychloroquine



gemifloxacin

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Table 1 | Pathological roles and functions of GSK3β in molecular pathways implicated in human cancer

Cancer type	Types of GSK3β inhibitors	*in vitro* or *in vivo*	Pathological roles for GSK3 and underlying mechanisms	Author [Ref.]
prostate	LiCl, SB216763 siRNA	*in vitro*	GSK3β renders prostate cancer cells resistant to TRAIL-induced apoptosis.	Liao X [64]
prostate	SB216763, SB415286, siRNA	*in vitro*	GSK3β maintains AR activity and prostate cancer cell proliferation.	Mazor M [65]
colorectal	AR-A014418 SB216763 shRNA, siRNA	*in vitro* tumor xenografts	Deregulated GSK3β expression and activity are associated with tumor cell survival and proliferation in CRC by mechanism independent of activation of Wnt/β-catenin signaling and Akt. GSK3β inhibition attenuates survival and proliferation of colon cancer cells by decreasing hTERT expression and telomerase activity and inducing cell senescence.	Shakoori A [57] Mai W [58, 60] Shakoori A [61]
colon	LiCl, TDZD8, SB216763, siRNA	*in vitro*	GSK3β functions against activation of p53-dependent apoptosis in colon cancer cells.	Ghosh JC [66]
colon	LiCl, SB216763, SB415286, LY2119301	*in vitro*	GSK3β functions against activation of p53-dependent apoptosis through a direct Bax-mediated mitochondrial pathway in colon cancer cells.	Tan J [67]
colon	LiCl, siRNA	*in vitro*	GSK3β functions against colon cancer cell apoptosis by inhibiting a TRAIL receptor-dependent synthetic lethal relationship between *Myc* activation and *FBW7* loss of function.	Rottmann S [68]
pancreatic	AR-A014418 SB216763, siRNA	*in vitro* tumor xenografts	GSK3β participates in NF-κB-mediated gene transcription and cell survival in pancreatic cancer cells. GSK3β inhibition attenuates proliferation of pancreatic cancer cell xenografts by inhibiting nuclear localization and activation of NF-κB.	Ougolkov A [69, 70]
pancreatic	AR-A014418 SB216763, siRNA	*in vitro*	Deregulated GSK3β expression and activity are associated with tumor cell survival and proliferation. GSK3β renders pancreatic cancer cells resistant to a chemotherapeutic agent (gemcitabine) by inhibiting p53 and c-Myc-mediated	Mai W [58, 60] Shimasaki T [62]

			pro-apoptotic pathways.	
stomach	AR-A014418 SB216763, siRNA	*in vitro*	Deregulated GSK3β expression and activity are associated with tumor cell survival and proliferation.	Mai W [58, 60]
liver	AR-A014418 SB216763, siRNA	*in vitro*	Deregulated GSK3β expression and activity are associated with tumor cell survival and proliferation.	Mai W [57, 59]
ovarian	LiCl	*in vitro* tumor xenografts	GSK3β positively regulates the proliferation of human ovarian cancer cells by increasing the expression of cyclin D1.	Cao Q [71]
oesophageal	LiCl	*in vitro*	GSK3β inhibition decreases the proliferation of a human oesophageal cancer cell line by inducing G_2/M cell cycle arrest.	Wang JS [72]
medullary thyroid	LiCl, SB216763	*in vitro* tumor xenografts	GSK3β is regulated by raf-1 pathway and is associated with proliferation of medullary thyroid cancer cells.	Kunnimalaiyaan M [73], Adler JT [74]
melanoma	LiCl, SB216763, DW1, DW2, DW1/2	*in vitro*	GSK3β functions against activation of p53-dependent apoptosis in human melanoma cells.	Smalley KS [75]
leukemia (AML)	LiCl, SB216763 TDZD8, 3-(3-carboxy-4-chloroanilino)-4-(3-nitrophenyl) maleimide	*in vitro*	GSK3β renders AML cells resistant to a chemotherapeutic agent (daunorubicin) by activating NF-κB.	De Toni F [76]
leukemia (B-cell CLL)	AR-A014418	*ex vivo*	Inhibition of GSK3β abrogates NF-κB binding to its target gene promoters through an epigenetic mechanism and enhances apoptosis in CLL B cells *ex vivo*.	Ougolkov AV [77]
leukemia	BIO	*in vitro* *ex vivo*	GSK3β inhibition suppresses leukemic cell growth via the induction of apoptosis mediated by down-regulation of survivin.	Holmes T [78, 79]
multiple myeloma	TDZD	*in vitro*	GSK3β enhances myeloma cell growth by phosphorylation of FOXO proapoptotic transcription factor resulting in its inactivation.	Zhou Y [80]

MLL leukemia	LiCl, SB216763 shRNA	*in vitro* tumor xenografts	GSK3β selectively maintains the survival and proliferation of *MLL* leukemia cells by decreasing $p27^{Kip1}$.	Wang Z [81]
glioma	LiCl, kenpaullone, LY2064827, 705701, 708244, 709125, shRNA	*in vitro* tumor xenografts	GSK3β inhibition leads to reduced glioma cell survival and clonogenicity by induction of c-Myc-dependent apoptosis, inactivation of intracellular NF-κB and alteration of intracellular glucose metabolism.	Kotliarova S [82]
glioma	LiCl, SB415286, AR-A014418, siRNA	*in vitro* *ex vivo*	GSK3β inhibitors decrease the migration of glioma cells *in vitro* and in brain tissue slices.	Nowicki MO [83]
glioblastoma multiforme	AR-A014418 SB216763, siRNA	*in vitro*	GSK3β inhibition attenuates the survival and proliferation of glioblastoma cells and sensitizes them to chemotherapeutic agents and ionizing radiation by activating p53-p21 and CDK6-Rb tumor suppressor pathways.	Miyashita K [59]
pheochromocytoma, paraganglioma	LiCl	*in vitro*	Treatment with lithium resulted in dose-dependent inhibition of GSK3β in tumor cells and reduced their proliferation.	Kappes A [84]

ADP, adenosine diphosphate; AML, acute myeloid leukemia; AR, androgen receptor; CDK/Cdk, cyclin-dependent kinase; CLL, chronic lymphocytic leukemia; CRC, colorectal cancer; FBW7, F-box/WD40 domain protein 7; FOXO, Forkhead box O; GSK3(β), glycogen synthase kinase 3(β); hTERT, human telomerase reverse transcriptase; MLL, myeloid/lymphoid or mixed lineage; NF-κB, nuclear factor-κB; shRNA, short hairpin RNA; siRNA, small interfering RNA; TRAIL, tumor necrosis factor (TNF)-related apoptosis-inducing ligand.

Table 2 | Proposed functions of GSK3β in the molecular pathogenesis of human cancers

Types of cells	Target molecules, cellular changes	Pathological roles for GSK3 and underlying mechanisms	Author [Ref.]
NS	Mdm2, p53, p21	GSK3β phosphorylates the central domain of Mdm2 resulting in Mdm2-dependent p53 degradation and decreased p21 expression.	Kulikov R [27]
NS	p53	ER stress induces p53 cytoplasmic localization (inactivation) and prevents p53-dependent apoptosis by a pathway involving GSK3β.	Qu L [28]
NS	$p21^{Cip1}$	GSK3β phosphorylates $p21^{Cip1}$ at Thr-57 residue within the Cdk binding domain and facilitates its degradation.	Rössig L [124]
glioblastoma	PTEN	GSK3 phosphorylates a tumor suppressor protein, PTEN, at Thr-366 residue leading to its destabilization.	Maccario H [28]
colon cancer	Hath1	GSK3β inhibits colonocyte differentiation by destabilizing the transcription factor, Hath1.	Tsuchiya K [118]
NS	JNK	GSK3β serves as a physiological switch to specifically repress JNK activation in response to LPA, sphingosine-1-phosphatase, or EGF.	Liu S [125]
NS	h-prune, cell motility/migration	GSK3β interacts with h-prune, cooperatively facilitating the disassembly of focal adhesions by activating FAK and Rac, and promoting cell migration.	Kobayashi T [115]
NS	Lamellipodia, cell migration	GSK3 enhances formation of long lamellipodia in human keratinocytes and cell migration.	Koivisto L [120]
NS	ADP-ribosylation factor 6, Rac1	GSK3 facilitates cell migration mediated by ADP-ribosylation factor 6 and Rac1 in response to hepatocyte growth factor/scatter factor.	Farooqui R [121]
NS	Rac1	GSK3β increases intestinal epithelial cell migration by activating Rac1 in response to Akt inhibition.	Vaidya RJ [122]

Cdk, cyclin-dependent kinase; EGF, epidermal growth factor; ER, endoplasmic reticulum; FAK, focal adhesion kinase; GSK3(β), glycogen synthase kinase 3(β); JNK, c-Jun N-terminal kinase; LPA, lysophosphatidic acid; Mdm 2, mouse double minute 2; NS, not specified; PTEN, phosphatase and tensin homologue deleted on chromosome 10;