Glycogen synthase kinase-3β is a pivotal mediator of cancer invasion and resistance to therapy

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Supplementary Table S1. Previous studies reporting the putative tumor suppressor roles of GSK3β.

Cancer type	Species	Summary of results	Effect of GSK3β inhibition on tumor cells*	Ref. No.
Colon	human	Stimulation of Wnt signaling by mutant K-ras^{Val12} was associated with inhibition of GSK3β activity in Caco-2 cancer cells.	Not examined	SR30
Stomach	human	Inhibition of GSK3β activity by pharmacological inhibitors induced expression of COX-2 mRNA and protein as well as the enzyme activity in TMK-1 and MKN-28 cancer cells.	Not examined	SR31
Pancreas	human	LiCl, GSK3β-siRNA or a kinase-dead mutant GSK3β transfection resulted in radioresistance of PANC-1 and BxPC-3 cancer cells, which was associated with stabilization of β-catenin and expression of its target gene.	GSK3β inhibition resulted in radio-resistance and its overexpression in radio-sensitization in cancer cells.	SR32
	human	Pancreatic cancer patients with higher expression of GSK3β in the tumors had a reduced risk of dying of pancreatic cancer.	Not examined	SR33
Liver	human	LiCl and SB-415286 repressed chemotherapeutic drugs induction of HepG2 cell apoptosis by inhibiting CD95 expression and caspase-8 activity and by disrupting nuclear GSK3β-p53 complexes.	GSK3β inhibitors render the cancer cells insusceptible to etoposide and camptothecin.	SR34
	human	PI3K inhibitor LY294002 sensitized HepB3 cells to etoposide and camptothecin by enhancing the expression of DR4 and DR5 and by decreasing pGSK3β^{S9}.	No direct effect was examined. SB-415286 repressed the chemosensitizing effect by LY294002 in the cancer cells.	SR35
	human	Decreased TSC2 and GSK3β expression in HCC tumors was significantly correlated with advanced clinico-pathological characteristics and poor prognosis of the patients.	Not examined.	SR36
	human	Overexpression of pGSK3β^{S9} in HCC tumors was significantly associated with the presence of type 2 DM and with poor prognosis of the patients.	Not examined.	SR37

	human	Ectopic expression of SIRT3 (a class III histone deacetylase) inhibited proliferation and inhibited apoptosis in HCC cells, which was associated with deacetylation of GSK3β and decreased $pGSK3\beta^{S9}$.	No direct effect was examined. GSK3β inhibitor reversed the SIRT3-induced proliferation inhibition and apoptosis in cancer cells.	SR38
Prostate	human	Transfection of wild-type and constitutively active mutant GSK3β repressed AR-mediated transactivation in cancer cells.	No direct effect was examined. Transfection of kinase-dead mutant GSK3β showed little effect on the AR transactivation in the cancer cells. LiCl abolished AR transactivation by GSK3β.	SR39
	human	A pharmacological GSK3β inhibitor, AR79, promotes cancer cell proliferation in soft tissue and bone in mice by dephosphorylation and stabilization of β-catenin.	GSK3β inhibitor promotes the cancer cell proliferation in mice.	SR40
Ovary	human	Level of $pGSK3\beta^{S9}$ but not total GSK3β and $pGSK3\beta^{Y216}$ was higher in cisplatin-resistant derivative of cancer cells than the parental cells.	No direct effect was examined. LiCl counteracted cisplatin-induced apoptosis in both parental and resistant cancer cells.	SR41
	human	Inhibition of GSK3β by SB-216763 increased MSX2 oncogenic factor via activation of β-catenin signaling in endometrioid cancer cells.	Not examined.	SR42
Uterine cervix (HeLa cells)	human	Inhibition of Akt enhances doxorubicin- or paclitaxel-induced apoptosis in cancer cells, which was associated with decrease in the level of $pGSK3\beta^{S9}$ and the binding of hexokinase II to mitochondria.	No direct effect was examined. GSK3β siRNA reversed the effect of Akt inhibitor on chemosensitivity of the cancer cells.	SR43
Breast	human	GSK3β inhibitors (LiCl, SB-216763 and SB-415286) decreased rapamycin-induced down regulation of cyclin D1, but not inhibit cell cycle G1 arrest in cancer cells. Rapamycin enhances paclitaxel-induced cytotoxicity in GSK3β wild-type but GSK3β-null cancer cells.	No direct effect was examined. GSK3β inhibition reversed rapamycin-induced down regulation of cyclin D1 expression in cancer cells.	SR44
	mouse	Transgenic mice overexpressing kinase-inactive GSK3β under the control of the mouse mammary tumor virus-long terminal repeat developed mammary tumors with overexpression of β-catenin and cyclin D1.	Not examined.	SR45

	human	Adiponectin attenuated cancer cell proliferation by suppression of Akt phosphorylation and pGSK3β^{S9} in association with accumulation and activation of β-catenin.	No direct effect was examined. LiCl reversed the effect of adiponectin in cancer cells.	SR46
	human	Therapeutic effect of prodigiosin, a bacterial metabolite, against cancer cells was associated with increased expression of NAG-1 via Akt dephosphorylation (inactivation).	No direct effect was examined. GSK3β inhibition with AR-A014418 reversed the effect of prodigiosin against the cancer cells.	SR47
	human	GSK3β phosphorylates Mcl-1 (proto-oncoprotein) for β-TrCP-mediated ubiquitination and proteasomal degradation in cancer cells.	Not examined.	SR48
	human	Expression of Mcl-1 was correlated with pGSK3β^{S9} in multiple cancer cell lines and primary cancer samples, and was significantly linked with poor prognosis of human breast cancer.	Not examined.	SR49
	human	GSK3β phosphorylates securin to promote its degradation via β-TrCP. A significant correlation between securin accumulation and pGSK3β^{S9} was observed in breast cancer tissues.	Not examined. Level of tumor pGSK3β^{S9} was correlated with Ki-67 proliferative index and tumor grades in breast cancer.	SR50
	mouse	Genetic deletion of GSK3 in mammary epithelial cells resulted in β-catenin activation and induced intraepithelial neoplasia that progressed to development of adenosquamous carcinoma. Mammary-specific knockout of GSK3 and β-catenin induced adenocarcinoma.	Not examined.	SR51
Lung	human	Constitutively active mutant GSK3β transfected in A549 cells binds to survivin, resulting in G1 cell-cycle arrest, apoptosis and sensitization to doxorubicin.	Dominant-negative mutant GSK3β and LiCl increased survivin expression, leading to cell-cycle progression and resistance to apoptosis.	SR52
	human	The level of pGSK3β^{S9} was associated with expression of Slug, a transcriptional repressor of E-cadherin, in cancer cells and non-small cell lung cancer. GSK3β-	Not examined.	SR53

		mediated phosphorylation of Slug facilitated Slug protein degradation.		
	human	Expression of a constitutively active GSK3β sensitized cancer cells to mTOR inhibitors. Higher basal levels of GSK3β activity in cancer cell lines correlated with more efficacious responses to the inhibitors.	No direct effect was examined. Pharmacologic inhibition and genetic depletion of GSK3β antagonized the effects of mTOR inhibitors against cancer cells.	SR54
Skin	mouse	The level of pGSK3β^{S9} was higher and that of pGSK3β^{Y216} was lower in the later stage of chemically-induced two-stage skin carcinogenesis mouse model.	Not examined.	SR55
	mouse	The level of pGSK3β^{S9} in skin carcinoma was weaker than normal skin. However, its level in TPA-mediated transformation-sensitive epidermal cells was higher than the transformation-resistant cells.	No direct effect was examined. Overexpression of wild-type and constitutively active mutant GSK3β in the TPA-mediated transformation-resistant epidermal cells suppressed EGF- and TPA-mediated anchorage-independent growth in soft agar and tumorigenicity in nude mice.	SR56
Melanoma	human	A multikinase inhibitor sorafenib activates GSK3β via inhibition of its upstream kinases and alters subcellular localization of p53 to induce apoptosis in B-raf mutant melanoma cells.	No direct effect was examined. GSK3β shRNA reversed and constitutively active mutant GSK3β facilitated the effect of sorafenib against tumor cells.	SR57
Neuroblastoma	human	BDNF activation of TrkB induced the Akt-dependent pGSK3β^{S9}, resulting in its inactivation. Treatment of neuroblastoma cells with inhibitors of GSK3β, LiCl, GSK3β inhibitor VII, kenpaullone, or a GSK3β-siRNA resulted in a 15% to 40% increase in neuroblastoma cell survival after treatment with etoposide or cisplatin.	GSK3β inhibition enhanced the survival of neuroblastoma cells after cytotoxic treatment.	SR58

*Direct effect of pharmacological GSK3β inhibitors and/or genetic depletion of GSK3β expression (e.g., RNA interference) or its activity (e.g., recombinant kinase-dead form) on tumor cell survival, proliferation, invasive ability and susceptibility to therapy.

Abbreviations: AR, androgen receptor; BDNF, brain-derived neurotropic factor; DM, diabetes mellitus; DR4, 5, death receptor 4, 5; EGF, epidermal growth factor; GSK3β, glycogen synthase kinase 3β; HCC, hepatocellular carcinoma; LiCl, lithium chloride (classical but not specific

GSK3β inhibitor); Mcl-1, myeloid cell leukemia-1; mTOR, mammalian target of rapamycin; MSX2, msh homeobox 2; NAG-1, nonsteroidal anti-inflammatory drug activated gene 1; pGSK3β^{S9}, GSK3β phosphorylated at seine 9 residue (inactive form); pGSK3β^{Y216}, GSK3β phosphorylated at tyrosine 216 residue (active form); PI3K, phosphatidylinositol 3-kinase; shRNA, short hairpin RNA; siRNA, small interfering RNA; SIRT3, sirtuin 3; TPA, 12-*O*-tetradecanoylpholbor-13-acetate; β-TrCP, β-transducin repeats-containing protein; TrkB, tyrosine kinase receptor B; TSC2, tuberous sclerosis protein 2;

Supplementary Table S2. Clinical trials of GSK3β inhibitors for treatment of diseases

GSK3β inhibitor (Company)	Disease	Trial ID and phase	Combined regimen	URL (access date: July 5, 2016)	Reference
AZD-1080 (AstraZeneca)	Alzheimer's disease	Phase I	none	https://ja.scribd.com/doc/851553/AstraZeneca-Therapy-R-D-Pipeline-Summary-December-7-2007	
NP031112/tideglusive (Noscira SA)	Progressive supranuclear palsy	NCT01049399 Phase IIb	none	https://clinicaltrials.gov/ct2/show/NCT01049399	SR60,61
	Alzheimer's disease	NCT01350362 Phase II	none	https://clinicaltrials.gov/ct2/show/NCT01350362	SR62,63
LY2090314 (Eli Lilly)	Acute leukemia	NCT01214603 Phase II	none	https://clinicaltrials.gov/ct2/show/NCT01214603	
	Metastatic pancreatic cancer	NCT01632306 Phase I/II	Gemcitabine, FOLFOX, or Gemcitabine + nab-paclitaxel	https://clinicaltrials.gov/ct2/show/NCT01632306	
	Advanced or metastatic solid cancer	NCT01287520 Phase I	Pemetrexed + carboplatin	https://clinicaltrials.gov/show/NCT01287520	SR64,65
CLOVA cocktail*	Advanced pancreatic cancer	UMIN000005095 Phase I/II	Gemcitabine	https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&action=brows&type=summary&recptno=R000006032&language=E	
	Recurrent glioblastoma	UMIN000005111 Phase I/II	Temozolomide	https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr.cgi?function=brows&action=brows&type=summary&recptno=R000002506&language=E	*Furuta T, et al.

Abbreviations: CLOVA, combined cimetidine, lithium chloride, olanzapine and valproate regimen; FOLFOX, combined folate, 5-fluorouracil and oxaliplatin regimen; SR, supplementary reference No.

*Furuta T, et al., unpublished data