# 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 |

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| | 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.<br>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.<br>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.<br>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.<br>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.<br>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 |

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| | 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.<br>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.<br>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.<br>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 |

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| | | 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