Issue Highlights

Phosphorylation-mediated regulation of GSK3β

Inhibition of Hedgehog pathway inhibits clonogenic growth of primary AML cells

Mechanisms of action of oncolytic virus therapy
Subcellular localization of CtIP and γH2AX. See also Wu, et al. (pages 1406–1415 of this issue).
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Glycogen synthase kinase-3β is a pivotal mediator of cancer invasion and resistance to therapy

Many types of human malignancies frustrate both clinicians and patients with their resistance to multimodal therapy and high rate of recurrence in spite of aggressive treatment. Research efforts have focused on identifying genetic and epigenetic patterns that may lead to resistance, and identifying a common link that defines resilience across cancer subtypes would be a major step forward in therapy. In this issue of *Cancer Science*, Domoto and colleagues provide a comprehensive review on glycogen synthase kinase-3β (GSK-3β) and its potential role as a driver of cancer invasiveness and resistance to therapy. The authors first provide the reader with a fundamental understanding of how cancers become invasive and then detail how GSK-3β functions in cells under normal and malignant conditions. The reader is left with a perspective on what next steps can be taken to better understand GSK-3β and how this may lead to novel anti-cancer agents.

doi: 10.1111/cas.13028

Small-molecule Hedgehog inhibitor attenuates the leukemia-initiation potential of acute myeloid leukemia cells

Aberrant activation of the Hedgehog (Hh) pathway is thought to be linked to a variety of cancers, including acute myeloid leukemia (AML), particularly in the role of maintaining the leukemic stem cell population; however, the particulars are not known. In this proof-of-concept study, Fukushima et al. attempted to determine how PF-0449913 (PF-913) works on the G-protein inhibitor of Hh called Smoothened in both *in vivo* and *in vitro* models of AML. They show that *in vivo* PF-913 works primarily on the quiescent cell population, causing minimal cell death, whereas *in vitro* reduced the tumor burden in a xenotransplant system, likely by working through inhibition self-renewal signatures and cell cycle progression. In addition, PF-913 served to sensitize leukemic cells to cytosine arabinoside. This exciting work serves to deepen the understanding of this novel drug and will hopefully guide treatment to improve outcomes for AML patients.

doi: 10.1111/cas.13019

Oncolytic virus therapy: a new era of cancer treatment at dawn

Cancer immunotherapy is an emerging field that has already revolutionized the way clinicians treat patients and researchers focus their efforts. In this timely review, Fukuhara et al. provide a summary of oncolytic virus therapy, a new cancer therapy that also utilizes host immunity, which seizes naturally occurring viruses and targets them to destroy cancer cells. The unique function of this therapy is that these viruses do not function as transgene carriers, but instead themselves are targeted anti-neoplastic agents. Local treatment with the viruses not only causes a robust viral replication and killing of cancer cells but also induces systemic antitumor immunity. The authors outline the long history of oncolytic virus therapy and describe how the field has been reborn, which has led to several recent approvals for novel cancer treatments. This review provides excellent groundwork for those interested in oncolytic virus therapy and simultaneously updates those in the field of the latest developments and emerging concepts.

doi: 10.1111/cas.13027
Glycogen synthase kinase-3β is a pivotal mediator of cancer invasion and resistance to therapy

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Key words
Cancer, GSK3β, invasion, therapeutic target, therapy resistance

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One of the well-recognized but still poorly understood characteristics of cancer is its ability to adapt and survive in a harsh microenvironment. Tumor cells survive the hypoxic and starved conditions by changing their morphological and biological properties and by interacting with tumor-associated stromal components.1-3 Another striking characteristic of cancers is their ability to invade host organs and to resist therapeutic insults, thus limiting the success of curative tumor resection and leading to tumor metastasis and therapy failure. These pathological behaviors of cancer are associated with acquisition of the hallmark biological traits of cancer.1-4 Despite recent advances in cancer treatments, the association of unresectable and recurrent cancers that share persistent capacity for invasion, metastasis and therapy resistance remains a challenge for current medical therapies.5,6 Although our knowledge of the molecular and biological mechanisms by which cancer evolves to the refractory stage is increasing,7,8 few strategies have been established to attenuate the ability of tumors to invade or to prevent the failure of therapy.

Modern options for cancer treatment consist of surgery, cytotoxic or cytostatic chemotherapeutics, radiation, molecular-targeted and immunomodulating agents, and their multidisciplinary combination.7,8 These treatments aim primarily to reduce tumor cell survival and proliferation, but not to eliminate invasive ability or resistance to therapy. Therefore, understanding the pathways by which cancer cells acquire both invasive and therapy-resistant phenotypes is critical for the development of more efficient therapeutic strategies against refractory cancers and, therefore, improvements in patient survival. Apart from the known mediators of invasion and therapy resistance [reviewed in Alexander and Friedl9], one emerging candidate is glycogen synthase kinase (GSK)-3β. This molecule has been extensively implicated in critical cell biology processes and has causal roles in common diseases including glucose intolerance, neurodegenerative disorders and cancer.10-12 Here we review previous studies that have reported on the association between therapeutic stimuli/resistance and induction of pro-invasive phenotypes in various cancer types. Many of these cancers have proven to be responsive to experimental treatment which targets GSK3β. This review focuses on the role of GSK3β as a molecular hub that connects pathways responsible for tumor invasion and resistance to therapy.
therapy, thus highlighting this kinase as a promising multipurpose cancer therapeutic target. It also discusses a putative role for GSK3β in sustaining the tumor cell “stemness” that is central to both tumor invasion and therapy resistance, thereby leading to intractable cancer and resulting in treatment failure.13–15

**Invasion and therapy resistance co-segregate in refractory cancer**

High invasiveness and resistance to therapy are common biological and clinical characteristics of refractory cancer that represent major challenges for research and treatment. The mechanisms involved in tumor invasion include cell motility and migration, degradation of the extracellular matrix and interaction with stromal and inflammatory cells. These are orchestrated in a way that enables the tumor to invade the host organ and often beyond.3,4 An early morphological and functional change for tumor cells of epithelial origin to acquire a proinvasive phenotype is epithelial to mesenchymal transition (EMT), altering cell behavior to resemble a mesenchymal type.16,17 The major modes by which cancers evade the effect of anti-cancer drugs include intrinsic (or constitutive) resistance and acquired resistance. Constitutive resistance to therapy may be due to mutational activation of signaling for cell survival, cytoprotective alterations in the cell cycle and in DNA repair ability, differences in the efficiency of drug uptake and efflux by cancer cells, and insufficient drug delivery [reviewed in Alexander and Friedl99]. Acquired resistance to chemotherapeutic and molecular-targeted agents involves distinct mechanisms, including genetic alterations to the drug targets, activation of surrogate pro-survival pathways, and interactions between tumor cells and components of the tumor environment [reviewed in Holohan et al.25, Ramos and Benetres-Alj60 and Alexander and Friedl99]. Intra-tumor heterogeneity emerges in many tumors and often underlies their resistance to therapeutic agents.18,19

The processes of tumor invasion and therapy resistance and their underlying mechanisms are often investigated as independent pathological events in cancer. However, recent studies (shown in Table 1) have demonstrated that tumor cells acquire morphological and functional proinvasive phenotypes with the ability to migrate and invade following the development of resistance to anti-cancer therapies (Suppl. References [SR] 1–24) (Data S1).1–24 The therapeutic modalities included conventional chemotherapeutic agents (SR1–15), different types of radiation therapy (SR16–20) as well as bioactive compounds targeting epidermal growth factor (EGF) receptor and vascular endothelial growth factor (VEGF).21–24 These studies indicate the therapeutic insult and/or resistance elicits proinvasive phenotypes and evokes the invasive capability of tumor cells in a broad spectrum of cancer types, including breast, colorectal, pancreatic, ovarian and prostate cancers, as well as rare intractable tumors (e.g. glioblastoma and osteosarcoma). A large number of preclinical studies have demonstrated that all available cancer treatments, including surgery, facilitate metastatic tumor spread. Such treatment often results in therapeutic benefit, but occasionally also results in resistance, leading to the paradoxical concept of “treatment-induced metastasis” [reviewed in Ebos20]. These experimental and preclinical studies suggest that the invasive behavior of cancer cells and their acquired resistance to therapy may not be separate pathological properties and could, instead, represent interconnected processes [reviewed in Alexander and Friedl99].

Regardless of the tumor type and therapeutic agent, the proposed mechanisms for invasion and resistance are attributable to molecular pathways that participate in tumor cell survival, transition to mesenchymal (EMT) and cancer stem cell (CSC) phenotypes, migration and invasion with extracellular matrix degradation, and drug efflux (Table 1). The reported molecules that mediate and interconnect these pathways include growth and transcription factors, chemokines, RAS and Rho family members (e.g. Ras, Rac1) and cell-matrix adhesion molecules (e.g. integrin family and focal adhesion kinase [FAK]) (Table 1). Bevacizumab is a humanized monoclonal antibody to VEGF used clinically as an anti-angiogenic agent. Acquired resistance to bevacizumab leads to enhanced tumor cell invasion due to the metabolic shift to glycolysis and degradation and remodeling of tumor stromal tissues.21–24 A better understanding of the biological mechanisms underlying the tight association between tumor invasion and therapy resistance should provide a solid rationale for the development of innovative cancer treatments.99

**Aberrant GSK3β in cancer**

GSK3 is a family of serine/threonine protein kinases comprising two highly conserved isoforms, GSK3α and GSK3β, that show approximately 85% overall homology and 98% homology in their kinase domains. GSK3 is constitutively active in normal cells and its activity is regulated by the differential phosphorylation at serine (S) residues 21 (pGSK3aS21) and 9 (pGSK3βS9) (both inactive forms), and tyrosine (Y) residues 279 (pGSK3aY279) and 216 (pGSK3βY216) (both active forms) (Fig. 1a,b). GSK3 regulates a diverse array of physiological cellular functions via the phosphorylation of and interaction with various proteins (Fig. 1c). Although the two GSK3 isoforms share many substrates, they are not functionally identical and show some differences in their substrate specificity [reviewed in Beurel et al.10]. Negative regulation of GSK3 activity is desirable to maintain physiological cellular functions. A growing number of studies have demonstrated that deregulated GSK3 activity contributes to the pathogenesis and progression of various diseases, including glucose intolerance, neurodegenerative disorders, and chronic inflammatory and immunological diseases.10,21 This points to GSK3 being an attractive and druggable target for a broad spectrum of diseases.22 Consequently, many GSK3 inhibitor compounds have been developed in academic and pharmaceutical institutes over recent years. Some are reported to inhibit both the α and β isoforms with different affinities,25–28 while others are specific for GSK3β (reviewed in SR25,26). Among the latter compounds, it was reported that AR-A014418 does not inhibit the activity of 26 closely related kinases and is, therefore, considered to be highly specific for GSK3β.29

Of the two GSK3 isoforms, most studies in the field of oncology have focused on GSK3β. This is partly because of the functional redundancy of the two isoforms in regulating the canonical Wnt/β-catenin pathway, responsible for generating the most prevalent oncogenic signaling.23,24 Based on its known effects on several proto-oncoproteins (e.g. β-catenin, cyclin D1 and c-Myc), cell cycle regulators (e.g. p27Kip1) and mediators of EMT (e.g. snail) (Fig. 1c), it has long been recognized that GSK3β suppresses tumor development [reviewed in Clevers and Nusse,25 Jope et al.26 Luo et al.,27], as discussed later (Table S1).30–38 Paradoxically, our earlier studies found increased expression and deregulated activity of GSK3β due to changes in the differential...
Table 1. Representative previous studies showing interrelationship between therapeutic stimuli/resistance and pro-invasive phenotype in cancer

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Therapeutic insults</th>
<th>Biological mechanism (Suppl. Reference number)</th>
<th>Therapeutic effect of GSK3β inhibition and underlying mechanism (Reference number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>Tamoxifen</td>
<td>EGFR pathway, enhanced tumor cell motility and invasion(\textsuperscript{SR1})</td>
<td>Suppression of invasion through dysregulation of actin-reorganization via down-regulation of WAVE2(\textsuperscript{68})</td>
</tr>
<tr>
<td></td>
<td>Tamoxifen</td>
<td>EMT induction with EGFR pathway-dependent β-catenin activation(\textsuperscript{SR2})</td>
<td></td>
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<tr>
<td></td>
<td>Adriamycin</td>
<td>Twist 1-mediated EMT induction and P-gp up-regulation(\textsuperscript{SR3})</td>
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<tr>
<td></td>
<td>Doxorubicin and cyclophosphamide</td>
<td>TNF-α/NF-κB-mediated amplification of CXCL1 paracrine network between carcinoma, myeloid, and endothelial cells(\textsuperscript{64})</td>
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<tr>
<td></td>
<td>alone or in combination</td>
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<tr>
<td>Colorectal cancer</td>
<td>Oxaliplatin</td>
<td>NF-κB-mediated EMT induction with enhanced cell migration and invasion(\textsuperscript{SR5})</td>
<td>Suppression of tumor cell survival and proliferation by inhibition of hTERT/ telomerase and promoting p53-dependent apoptosis(\textsuperscript{28–32,33}) and invasion by down-regulation of WAVE2(\textsuperscript{68}) and enhancing 5-FU effect via PARP1-dependent and AIF-mediated necroptosis(\textsuperscript{73})</td>
</tr>
<tr>
<td></td>
<td>Erlotinib</td>
<td>EMT induction(\textsuperscript{SR21})</td>
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<tr>
<td>Pancreatic cancer</td>
<td>Gemcitabine</td>
<td>EMT induction with activation of β-catenin and c-Met and acquisition of CSC phenotype(\textsuperscript{SR6})</td>
<td>Suppression of tumor cell survival and proliferation(\textsuperscript{30}) by inhibition of NF-κB transcriptional activity(\textsuperscript{34,35}) synergistic with gemcitabine by restoration of Rb function(\textsuperscript{60}) and inhibition of TP53INP1-mediated DNA repair(\textsuperscript{34,35}) increase in radiosensitivity and suppression of invasion via FAK/Rac1/MMP-2 and CXCR4/MMP-2 pathways(\textsuperscript{60,67})</td>
</tr>
<tr>
<td></td>
<td>Gemcitabine</td>
<td>Acquisition of EMT and CSC phenotypes with activation of Notch pathway(\textsuperscript{SR7})</td>
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<tr>
<td></td>
<td>Gemcitabine, 5-FU, cisplatin</td>
<td>Gene expression profile responsible for EMT phenotype(\textsuperscript{SR8})</td>
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<tr>
<td></td>
<td>Gemcitabine</td>
<td>NF-κB-mediated acquisition of EMT and CSC phenotypes(\textsuperscript{SR9})</td>
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<td></td>
<td>γ-irradiation</td>
<td>Tumor cell migration and invasion with enhanced MMP-2 activity(\textsuperscript{SR16})</td>
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<tr>
<td>Ovarian cancer</td>
<td>Erlotinib</td>
<td>EMT induction(\textsuperscript{SR21})</td>
<td>Suppression of tumor cell proliferation by decrease in cyclin D1 expression(\textsuperscript{39})</td>
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<tr>
<td></td>
<td>Taxol, vincristine</td>
<td>Increased expression of twist(\textsuperscript{SR10})</td>
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<td></td>
<td>Paclitaxel</td>
<td>Acquisition of EMT and metastatic potential(\textsuperscript{SR11})</td>
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<td></td>
<td>Cisplatin, taxol</td>
<td>Acquisition of EMT via endothelin A receptor-mediated pathway(\textsuperscript{SR12})</td>
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<tr>
<td>Prostate cancer</td>
<td>Taxol, vincristine</td>
<td>Increased expression of twist(\textsuperscript{SR10})</td>
<td>Suppression of tumor cell survival and proliferation by eliminating TRAIL resistance, repressing AR activity(\textsuperscript{41–44}) and attenuation of metastasis by depleting CSC population(\textsuperscript{78})</td>
</tr>
<tr>
<td></td>
<td>Docetaxel</td>
<td>Gene expression profile responsible for EMT phenotype(\textsuperscript{SR13})</td>
<td></td>
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<tr>
<td>Other epithelial cancer</td>
<td>Ionizing radiation</td>
<td>EMT induction with enhanced cell migration(\textsuperscript{SR17})</td>
<td>Suppression of tumor cell survival by interrupting ERK-mediated prosurvival pathway(\textsuperscript{46})</td>
</tr>
<tr>
<td>Endometrial</td>
<td>Taxol, vincristine</td>
<td>Increased expression of twist(\textsuperscript{SR10})</td>
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<tr>
<td>Bladder</td>
<td>Cisplatin</td>
<td>EMT induction and enhanced cell migration and invasion in association with increased BMI1 by down-regulation of miR-200b and miR-155(\textsuperscript{SR14})</td>
<td></td>
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<tr>
<td>Tongue</td>
<td>Taxol, vincristine</td>
<td>Increased expression of twist(\textsuperscript{SR10})</td>
<td>Not shown</td>
</tr>
<tr>
<td>Nasopharyngeal</td>
<td>Taxol, vincristine</td>
<td>Increased expression of twist(\textsuperscript{SR10})</td>
<td>Not shown</td>
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</table>
Table 1 (Continued)

<table>
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<tr>
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<tr>
<td>Glioblastoma</td>
<td>Doxorubicin</td>
<td>Therapeutic effect and enhancement of doxorubicin effect by a new anti-inflammatory small molecule (B) ( ^{SR15} )</td>
<td>Suppression of tumor cell survival and proliferation by restoring p53/p21 pathway and inhibition of c-Myc, NF-κB and abnormal glycolysis, ( ^{31,36} ) synergistic with temozolomide and increase in radiosensitivity via Rb-mediated and c-Myc/DMNT3A/MGMT pathways, ( ^{31,69} ) suppression of invasion ( ^{58,59,61} ) by inhibition of FAK/Rac1/JNK pathway ( ^{61} ) and induction of CSC differentiation ( ^{33,77} )</td>
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<td></td>
<td>Sublethal irradiation</td>
<td>Enhanced tumor cell migration and invasion involving αvβ3 integrin, MMP-2, MMP-9, MT1-MMP, TIMP-2 and BCL-2/BAX rheostat ( ^{SR18} )</td>
<td>Suppression of tumor cell survival and proliferation by restoring p53/p21 pathway and inhibition of c-Myc, NF-κB and abnormal glycolysis, ( ^{31,36} ) synergistic with temozolomide and increase in radiosensitivity via Rb-mediated and c-Myc/DMNT3A/MGMT pathways, ( ^{31,69} ) suppression of invasion ( ^{58,59,61} ) by inhibition of FAK/Rac1/JNK pathway ( ^{61} ) and induction of CSC differentiation ( ^{33,77} )</td>
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<tr>
<td></td>
<td>Ionizing radiation</td>
<td>Enhanced tumor cell migration with increased expression of β3 and β1 integrins ( ^{SR19} )</td>
<td>Suppression of tumor cell survival and proliferation by restoring p53/p21 pathway and inhibition of c-Myc, NF-κB and abnormal glycolysis, ( ^{31,36} ) synergistic with temozolomide and increase in radiosensitivity via Rb-mediated and c-Myc/DMNT3A/MGMT pathways, ( ^{31,69} ) suppression of invasion ( ^{58,59,61} ) by inhibition of FAK/Rac1/JNK pathway ( ^{61} ) and induction of CSC differentiation ( ^{33,77} )</td>
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<tr>
<td></td>
<td>Bevacizumab</td>
<td>Resistance to the therapy is associated with up-regulation of MMP-2, MMP-9, MMP-12, TIMP1, SPARC and HIF-2α, and with activation of bFGF-mediated alternate angiogenesis pathway ( ^{GR22} )</td>
<td>Suppression of tumor cell survival and proliferation by inhibition of NF-κB transcriptional activity ( ^{38} ) and restoring β-catenin osteosarcoma suppressor (S. Shimozaki et al.) ( ^{†} )</td>
</tr>
<tr>
<td></td>
<td>Bevacizumab</td>
<td>Treatment of tumor xenograft is associated with decrease of mitochondria, induction of glycolytic metabolites (lactate and alanine) and HIF-1α, and activation of PI3K pathway ( ^{GR23} )</td>
<td>Suppression of tumor cell survival and proliferation by inhibition of NF-κB transcriptional activity ( ^{38} ) and restoring β-catenin osteosarcoma suppressor (S. Shimozaki et al.) ( ^{†} )</td>
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<tr>
<td></td>
<td>Bevacizumab</td>
<td>Resistance to the therapy induced genes associated with a mesenchymal origin, cellular migration/invasion, and inflammation ( ^{GR24} )</td>
<td>Suppression of tumor cell survival and proliferation by inhibition of NF-κB transcriptional activity ( ^{38} ) and restoring β-catenin osteosarcoma suppressor (S. Shimozaki et al.) ( ^{†} )</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td>Low-dose photon irradiation</td>
<td>Enhanced cell migration and invasion concomitant with up-regulation of αvβ3 integrin ( ^{GR20} )</td>
<td>Suppression of tumor cell survival and proliferation by inhibition of NF-κB transcriptional activity ( ^{38} ) and restoring β-catenin osteosarcoma suppressor (S. Shimozaki et al.) ( ^{†} )</td>
</tr>
</tbody>
</table>

†S. Shimozaki et al., unpublished observation. AIF, apoptosis-inducing factor; AR, androgen receptor; BAX, Bcl-2-associated X protein; bFGF, basic fibroblast growth factor; BM1, B lymphoma Mo-MLV insertion region 1 homolog; CSC(s), cancer stem-like cell(s); CXCL1, chemokine (C-X-C motif) ligand 1; CXC4, CXC receptor type 4; DMNT3A, DNA (cytosine-5)-methyltransferase 3A; EGF, epidermal growth factor receptor; EMT, epithelial-mesenchymal transition; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; S-FU, 5-fluourouracil; HIF, hypoxia inducible factor; hTERT, human telomerase reverse transcriptase; JNK, c-Jun N-terminal kinase; MGMT, O6-methylguanine DNA methyltransferase; miR, micro-RNA; MMP, matrix metalloproteinase; MT1-MMP, membrane type 1-MMP; NF-κB, nuclear factor-κB; PARP1, poly [ADP-ribose] polymerase 1; P-gp, P-glycoprotein (multidrug resistance); PI3K, phosphatidylinositol-3-kinase; P-m, phosphorylation of the specified residue; Rb, retinoblastoma; SPARC, secreted protein, acidic, cysteine rich; SR, supplementary reference No.; TIMP, tissue inhibitor of MMP; TNF-α, tumor necrosis factor-α; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; WAVE2, WAS (Wiskott-Aldrich syndrome) protein family member 2.

Phosphorylation of S9 and Y216 residues in gastrointestinal cancers, glioblastoma and osteosarcoma compared to respective normal cells and tissues. \( ^{25–31} \) These observations suggested that GSK3β could participate in cancer development and progression, despite the general belief that it has tumor-suppressive functions. \( ^{25–27} \) We and other research groups have shown that inhibition of GSK3β activity with specific pharmacological inhibitors, or inhibition of its expression by RNA interference, can preferentially attenuate the survival and proliferation of tumor cells and induce them to undergo apoptosis. This effect has been observed not only in gastrointestinal and pancreatic cancer cells \( ^{28–32,35} \) but also in glioblastoma \( ^{31,36,37} \) and osteosarcoma (S. Shimozaki, N. Yamamoto, T. Domoto, H. Nishida, K. Hayashi, H. Kimura, A. Takeuchi, S. Miwa, K. Igarashi, T. Kato, Y. Aoki, T. Higuchi, M. Hirose, R.M. Hoffman, T. Minamoto, H. Tsuchiya, unpublished data, 2016) \( ^{38} \) and other malignant neoplasms such as gynecological and urogenital cancers \( ^{39–47} \), soft tissue sarcomas \( ^{48,49} \), hematological malignancies \( ^{50,51} \) and lung cancers \( ^{52,53} \). This accumulating evidence firmly establishes GSK3β as a valuable target in cancer treatment. \( ^{11,12} \)

There has been substantial interest in the molecular mechanisms by which GSK3β favors tumor progression and by which inhibition of its activity or expression attenuates tumor cell survival, immortalization and proliferation. The reported mechanisms for tumor cell survival include the nuclear factor (NF)-κB-mediated prosurvival pathway \( ^{14–16} \), tumor cell resistance to apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) \( ^{41} \) and failure of the p53-mediated tumor suppressor pathway or of the Rb-mediated cell...
Preservation of telomere length by maintaining activity of human telomerase reverse transcriptase (hTERT) and telomerase also immortalizes tumor cells in response to the aberrant activation of GSK3β. (31,32) Cell proliferation pathways mediated by c-Myc and cyclin D1 sustain tumor cell proliferation that is dependent on GSK3β. (33,34) In osteosarcoma and rhabdomyosarcoma, the induction of β-catenin signaling, a well-known tumor suppressive mechanism in these cancers, has been linked to decreased cell proliferation following GSK3β inhibition. (S. Shimozaki et al., unpublished data). Another recent study reported that mitotic catastrophe caused by disruption of centrosome biodynamics was associated with attenuated tumor cell survival and proliferation following GSK3β inhibition. (54) Importantly, GSK3β has little impact on the cell survival, immortalization and growth of normal cells, where the differential phosphorylation of S9 and Y216 residues functions to fine tune GSK3β activity. (28–31,34) The growing body of evidence on the role of aberrant GSK3β in sustaining and promoting cancer cell survival, immortalization and proliferation, together with the differential effects of GSK3β inhibition on cancer and normal cells, underpins the targeting of GSK3β as a novel cancer treatment. (31,32)

GSK3β mediates both the invasive phenotype and therapy resistance in refractory cancer

The dependency of cancer cells on GSK3β for their survival and proliferation has encouraged further studies on whether aberrant GSK3β participates in tumor cell invasion and therapy resistance, the two major determinants of patient outcome. (3–6) As shown in Table 1, most tumors that acquire pro-invasive phenotypes as they evade therapy are susceptible to experimental treatment involving inhibition of GSK3β. Here we review what is known about the involvement of GSK3β in tumor invasion and resistance to therapy based on our previous studies in pancreatic cancer and glioblastoma. These are representative of lethal tumors and are characterized by high invasive capacity and resistance to available therapies. (55,56) GSK3β and cancer invasion. While GSK3β plays pivotal roles in cytoskeletal organization, cell polarity and migration in the physiological processes of organogenesis and wound healing, (57) little is known about its role in cancer cell motility, migration and invasion. Earlier studies showed that lithium chloride and some indirubins, both classical GSK3-inhibiting agents, inhibited the migration and invasion of glioblastoma.

Fig. 1. (a) Comparison of the structural and functional domains of the two GSK3 isoforms, (b) the sites (S9 and Y216) of phosphorylation of GSK3β by different kinases regulating GSK3β activity, and (c) the substrates of GSK3β and proteins that interact with it. (a) GSK3α (51 kD) and GSK3β (47 kD) are products of their respective genes located in chromosomes 19q13 and 3q13. The isoforms share high (98%) homology of the catalytic domains, and GSK3α has a glycine-rich extension at the N-terminal side. Blue and red narrow columns indicate the sites of serine (S) and tyrosine (Y) phosphorylation, respectively. (b) The kinases indicated in blue phosphorylate GSK3β-S9 resulting in its inactivation, while those indicated in red phosphorylate GSK3β-Y216 resulting in its activation. (c) GSK3β stabilizes/activates (red arrows) and destablizes/inactivates (blue lines) various transcription factors as well as structural and functional proteins. AP-1, activator protein 1; APC, adenomatous polyposis coli; BAX, BCL2-associated X protein; BAX, B-cell lymphoma; C, C-termianal of protein; CEBP, CCAAT (cytosine-cytosine-adenosine-adenosine-thymidine)-enhancer-binding protein; cdc25A, cell division cycle 25 homolog; CREB, cAMP (cyclic adenosine monophosphate) response element binding protein; CRMP2, collapsin response mediator protein 2; eIF2B, eukaryotic initiation factor 2B; FAK, focal adhesion kinase; FGDD-1/3, FYVE RhoGDP guanine nucleotide exchange factor; FKHR, forkhead in rhabdomyosarcoma; Gly, glycine; GR, glucocorticoid receptor; GS, glycogen synthase kinase 3; HIF-1α, hypoxia inducible factor-1α; HSF-1, heat shock transcription factor-1; ILK, integrin-linked kinase; IPF1/PDX1, insulin promoter factor 1/pancreatic and duodenal homeobox 1; IRS1, insulin receptor substrate 1; LRP5/6, lipoprotein receptor-related protein 5/6; MFA, musculoaponeurotic fibrosarcoma oncogene homolog A; MAP1B/2C, microtubule associated protein 1B/2C; Mcl1, myeloid cell leukemia 1; mCRY2, mouse cryptochrom 2; MDM2, mouse double minute 2 homolog; MEK, MAPK (mitogen-activated protein kinase)/ERK (extracellular signal-regulated kinase) kinase; MITF, microphthalmia-associated transcription factor; MLK3, mixed lineage kinase 3; N, N-terminal of protein; NAC-α, nascent polypeptide-associated complex subunit-α; NF-AT, nuclear factor of activated T-cells; NF-κB, nuclear factor-κB; Nrf2, nuclear factor erythroid 2-related factor 2; p130RB, p130 retinoblastoma; p21CIP1, p21 CDK (cyclin-dependent kinase)-interacting protein 1; PPAR, peroxisome proliferator-activated receptor; PTEN, phosphatase and tensin homolog; Pyk-2, proline-rich tyrosine kinase 2; RAR, retinoic acid receptor; Red1, RNA-editing deaminase 1; S, serine; SRC-3, steroid receptor coactivator-3; SREBP, sterol regulatory element-binding protein; TSC2, tuberous sclerosis complex 2; VDAC, voltage-dependent anion channel; Y, tyrosine.
invasion via the promotion of morphological and functional pro-inflammatory changes in the tumor microenvironment. Our observations therefore suggest that GSK3β kinase (JNK). This results in decreased expression of matrix metalloproteinase (MMP)-2 and membrane type (MT)-1-MMP.

Our observations therefore suggest that GSK3β sustains tumor invasion via the promotion of morphological and functional pro-inflammatory changes in the tumor microenvironment. This is particularly relevant for the treatment of refractory cancer and, hence, molecular-targeted therapy is often combined with conventional chemotherapeutics and/or radiation therapy, as well as with other targeted agents. As discussed above, accumulating evidence on the effects of GSK3β inhibition against cancer cell survival and proliferation has led us to address whether this could be used in combination with chemotherapy and irradiation.

The standard treatment modalities prescribed are often ineffective in patients with refractory tumors such as pancreatic cancer and glioblastoma.

Other recent studies have also reported that GSK3β inhibition allows therapy-resistant colon and pancreatic cancer cells to become susceptible to 5-fluorouracil (5-FU), and renal cell carcinoma cells to become susceptible to a synthetic multikinase inhibitor (sorafenib) that targets growth signaling and angiogenesis. Together, these studies suggest that GSK3β participates in multiple molecular pathways used by various cancer types to evade chemotherapy, radiotherapy and targeted therapies.

Collectively, a growing body of experimental evidence supports the notion that GSK3β is a molecular “hub” that mediates and connects various pathways responsible for tumor invasion and resistance to therapy. Together with the role of GSK3β in promoting tumor cell survival and proliferation and its differential functions between tumor and normal cells [reviewed in Miyashita et al.(11) and McCubrey et al.(12)], this evidence reinforces the promise of novel cancer therapeutic strategies that target GSK3β.

Future perspectives

Targeting of the biological pathways that link tumor invasion and therapy resistance is clearly an attractive prospect for the...
Fig. 3. (a) Putative molecular pathway that links GSK3β activity with the resistance of pancreatic cancer cells to DNA damage induced by gemcitabine and ionizing radiation. The effects of GSK3β on E2F-dependent gene transcription and on the expression of RR, TS and TK remain to be determined. CDK, cyclin-dependent kinase; E2F, E2 factor; circled P, phosphorylation; Rb, retinoblastoma (tumor suppressor protein); RR, ribonucleotide reductase; TK, thymidine kinase; TS, thymidine synthase. (b) Regulation of MGMT expression by GSK3β signaling in glioblastoma. GSK3β inhibition results in c-Myc activation directly and via activation of β-catenin-mediated signaling, which consequently increases recruitment of DNMT3A by c-Myc to the MGMT promoter, thus increasing de novo DNA methylation in the MGMT promoter. The methylated status of the MGMT promoter increases the sensitivity of glioblastoma to temozolomide.

Fig. 4. Involvement of GSK3β in the representative pathological hallmarks of cancer. GSK3β positively regulates the distinct molecular pathways and participates in survival, proliferation, migration and invasion of tumor cells and their insensitivity and resistance to cancer therapy. The cancer stemness phenotypes might underlie the process of these pathological hallmarks. Abbreviations are defined in Figures 1, 2 and 3.

GSK3β has pivotal functions in normal cell biology and is also central to the processes of cancer cell survival, proliferation, invasion and therapy resistance, as discussed above. A number of studies have reported that CSC phenotypes share unique molecular pathways and tumor-environment interactions that are also associated with tumor invasion and therapy resistance. This leads us to propose a working hypothesis whereby GSK3β underlies the basal mechanism for sustaining the CSC phenotype (Fig. 4). Recent studies have shown that GSK3β negatively controls the differentiation of malignant glioma cells, and that GSK3β inhibition results in depletion of cancer stem-like or progenitor-like cells and attenuation of metastatic spread in prostate cancer. Consistent with the physiological roles of GSK3β in negatively regulating canonical Wnt/β-catenin and hedgehog signaling, inhibition of GSK3β is a prerequisite for the maintenance of “stemness” phenotypes in embryonic and hematopoietic stem cells. Therefore, future work should aim to understand the influence of GSK3β on both tumor and normal stem cell phenotypes. This knowledge can be applied for the development of novel cancer treatments that target GSK3β.

One of the concerns about targeting GSK3β for cancer treatment is that its inhibition may promote the progression of existing tumors [reviewed in Takahashi-Yanaga, Luo and McCubrey et al.]. A number of studies have focused on the putative tumor suppressive role of GSK3β (Table S1). Many of these report inactivation or activation of GSK3β as a mediating event in pathways leading to tumor progression or suppression, respectively. An inverse association between tumor expression of GSK3β and the survival of cancer patients has been reported. Other studies found causal links between pharmacological GSK3β inhibition and/or depletion of GSK3β expression (e.g. gene knockout and RNA interference) or activity (e.g. recombinant kinase-dead or constitutively active mutant form) and tumor cell survival, proliferation and susceptibility to cancer therapies. GSK3β inhibition strategies and the development of GSK3β-targeted agents therefore require careful evaluation to determine whether the tumor promoting function of GSK3β is counteracted by its putative tumor suppressor function in different cancer types. The development and administration of GSK3β inhibitors for the treatment of chronic diseases, including diabetes...
mellitus, neurodegenerative disorders and cancer, requires serious awareness of the safety issues. A major concern is whether and how GSK3β can be targeted to treat disease, because its systemic inhibition may lead to unwanted effects by disrupting multiple biological pathways (Fig. 1c). Most notably, long-term inhibition of GSK3β may initiate tumorigenesis due to its role in suppressing proto-oncogenic pathways, in particular that are mediated by β-catenin [reviewed in Takahashi-Yanaga(22), Jope et al. (23,24) Luo(25) and McCubrey et al. (27)]. A previous study showed that genetic deletion of GSK3β in mammary epithelial cells resulted in β-catenin activation and induced intraepithelial neoplasia that progressed to the development of adenocarcinoma (SR57). Moreover, overexpression of wild-type and constitutively active mutant GSK3β in 12-O-tetradecanoylphorbol-13-acetate (TPA)-mediated, transformation-resistant mouse epidermal cells suppressed EGF-mediated and TPA-mediated anchorage-independent growth in soft agar and tumorigenicity in rodents (Table S1). (SR58) However, there is currently no direct evidence to support tumor development in vivo following treatment with a GSK3β inhibitor [reviewed in Miyashita et al. (11) and SR27]. As discussed in previous studies that report cancer therapeutic effects of GSK3β inhibition (Table 1), none of the available experimental GSK3β inhibitors induces neoplastic transformation of NIH 3T3 nontransformed normal cells or tumor development in experimental animal models [reviewed in Miyashita et al. (11)] and SR27]. Long-term prescription of lithium, the only GSK3β inhibitor approved for the treatment of bipolar disorder since the 1950s, has not been associated with an increased risk of cancer or death from cancer. (SR59) Post-translational regulation of GSK3β activity via the phosphorylation of S9 and Y216 (pGSK3βS9 versus pGSK3βY216), (Fig. 1b) in response to various stimuli could partly underlie a mechanism that protects normal cells from the detrimental effects of GSK3β inhibition.

Despite the concerns outlined above, clinical trials for neurodegenerative diseases and cancer have tested some seed pharmacological GSK3β inhibitor compounds and also approved medicines with the ability to inhibit GSK3β activity (Table S2). The former trials include AZD-1080 (AstraZeneca) for the treatment of Alzheimer’s disease (phase I), and NP031112 (tideglulsive; Noscira SA) for the treatment of progressive supranuclear palsy (NCT01049399; phase II), SR62,63) and of Alzheimer’s disease (NCT01350362; phase II). (SR60,61) Numerous studies have shown that, in addition to differential phosphorylation at S9 and Y216 residues (Fig. 1a,b), the subcellular localization of GSK3β together with various upstream pathways can regulate the expression and activity of this kinase [reviewed in Beurel et al. (10)] and Takahashi-Yanaga(22)]. New classes of GSK3β inhibitors that spatially and temporally control the expression and activity of GSK3β may therefore be required to reduce the risk of adverse events such as carcinogenesis that may be associated with long-term GSK3β inhibition.

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Disclosure Statement

The authors have no conflict of interest to declare.
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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Previous studies reporting the putative tumor suppressor roles of GSK3\(\beta\).

Table S2. Clinical trials of GSK3\(\beta\) inhibitors for treatment of diseases.

Data S1. Supplementary references.