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GSK3β as a novel promising target to overcome chemoresistance in pancreatic cancer

Camilla Pecoraro [a,b,1], Beatrice Faggion [a,1], Beatrice Balboni [a,c], Daniela Carbone [b], Godefridus J. Peters [a,d], Patrizia Diana [b], Yehuda G. Assaraf [e], Elisa Giovannetti [a,f,*]

[a] Department of Medical Oncology, Amsterdam University Medical Center, VU University, 1081 HV Amsterdam, the Netherlands
[b] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy
[c] Computational and Chemical Biology, Istituto Italiano di Tecnologia, Via Morego 30, 16163 Genoa, Italy, and Department of Pharmacy and Biotechnology, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy
[d] Department of Biochemistry, Medical University of Gdansk, Poland
[e] The Fred Wyszkowski Cancer Research Laboratory, Department of Biology, Technion-Israel Institute of Technology, Haifa, 3200003, Israel
[f] Cancer Pharmacology Lab, Fondazione Pisana per la Scienza, Via Ferruccio Giovannini 13, 56017 San Giuliano Terme (Pisa), Italy

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ABSTRACT

Pancreatic cancer is an aggressive malignancy with increasing incidence and poor prognosis due to its late diagnosis and intrinsic chemoresistance. Most pancreatic cancer patients present with locally advanced or metastatic disease characterized by inherent resistance to chemotherapy. These features pose a series of therapeutic challenges and new targets are urgently needed.

Glycogen synthase kinase 3 beta (GSK3β) is a conserved serine/threonine kinase, which regulates key cellular processes including cell proliferation, DNA repair, cell cycle progression, signaling and metabolic pathways. GSK3β is implicated in non-malignant and malignant diseases including inflammation, neurodegenerative diseases, diabetes and cancer. GSK3β recently emerged among the key factors involved in the onset and progression of pancreatic cancer, as well as in the acquisition of chemoresistance. Intensive research has been conducted on key oncogenic functions of GSK3β and its potential as a druggable target; currently developed GSK3β inhibitors display promising results in preclinical models of distinct tumor types, including pancreatic cancer.

Here, we review the latest findings about GSK-3β biology and its role in the development and progression of pancreatic cancer. Moreover, we discuss therapeutic agents targeting GSK3β that could be administered as monotherapy or in combination with other drugs to surmount chemoresistance. Several studies are also defining potential gene signatures to identify patients who might benefit from GSK3β-based therapeutic intervention. This detailed overview emphasizes the urgent need of additional molecular studies on the impact of GSK3β inhibition as well as structural analysis of novel compounds and omics studies of predictive biomarkers.

1. Introduction

Pancreatic cancer is currently the third most common cancer in Western countries, with an increasing incidence and poor outcome and constitutes one of the most lethal of the common malignancies with a poor five-year survival rate below 10 % (Hill and Chung, 2020; Siegel et al., 2021; Rahib et al., 2014; Coppola et al., 2017; Binenbaum et al., 2015)

Pancreatic ductal adenocarcinoma (PDAC) accounts for approximately 90 % of pancreatic tumors (Sarantis et al., 2020). This malignancy is among one of most inadequately understood human disorders, posing a significant diagnostic and therapeutic challenge. The lack of specific symptoms and reliable biomarkers for early detection screening of asymptomatic PDAC patients, results in a most dismal prognosis (Kaur et al., 2017; Zhou et al., 2017). In this respect, approximately 52 % of PDAC patients are diagnosed with an advanced-stage or metastatic disease, for which the 5-year survival trend is as low as 3% (Giovannetti et al., 2017; Supadmanaba et al., 2021).

The aggressive nature and the early metastatic behavior of PDAC are

* Corresponding author at: Department of Medical Oncology, Amsterdam University Medical Center, VU University, 1081HV Amsterdam, the Netherlands.
E-mail address: elisa.giovannetti@gmail.com (E. Giovannetti).
[1] Co-first authors.

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not the only responsible factors for the poor prognosis of this disorder, but also for its insensitivity to most therapies, such as chemotherapy, radiotherapy and immunotherapy. Among all clinical intervention, surgical resection remains the mainstay chance for cure. However, less than 20 % of patients are good candidates for pancreatectomy due to the usually diagnosed metastatic state of PDAC (Giovannetti et al., 2017). Additionally, chemotherapeutic and radiotherapeutic regimens are often palliative and their high toxicity provides a very marginal improvement in the survival rate of patients with advanced disease (Zeng et al., 2019). This poor treatment efficacy is accompanied by either intrinsic resistance or rapid acquisition of chemoresistance (Arora et al., 2013; Caparello et al., 2016). Thus, despite the advances in the use of combination chemotherapeutic regimens, survival remains dismal, highlighting the tremendous urgency for the design and development of novel therapeutic strategies to overcome the chemoresistant nature of this lethal disease.

In recent years, GSK3β has emerged as a new potential target in PDAC due to its involvement in promoting neoplastic transformation, tumor cell survival and chemoresistance (Cormier and Woodgett, 2017; Ding and Billadeau, 2020; Uehara et al., 2020). Clinical trials are currently testing several GSK3β inhibitors either as monotherapy or in combination with chemotherapeutic agents with the aim of developing promising PDAC therapeutic interventions that suppress PDAC growth and prevent disease progression (Garcia-Sampedro et al., 2021; Abrams et al., 2021).

The purpose of the current review is to determine whether or not GSK3β might be considered a good therapeutic target in advanced PDAC and which patient signatures might be prognostic of good therapy response. Furthermore, it will focus on GSK3β inhibitors that are currently approved or are undergoing clinical trials. We also discuss possible drug combinations that might prevent tumor recurrence and therapy resistance.

2. Glycogen synthase kinase 3 (GSK3)

2.1. GSK3β biology in normal cells

Glycogen synthase kinase 3 (GSK3) is a family of serine-threonine kinases which encompasses two highly conserved isoforms, GSK3α and GSK3β, sharing approximately 85 % overall sequence homology (Woodgett, 1990). Even if functional redundancy has been observed within the two isoforms, most studies in the oncology field focused on GSK3β activity, mainly due to its known enigmatic effects on many physiological and pathological processes. By phosphorylating serine and threonine residues of a broad range of functional and structural proteins, GSK3β regulates many fundamental biological processes in cells such as glycogen metabolism, Wnt/β-catenin signaling, G-protein-coupled signal transduction and maintenance of stem cell identity (Cormier and Woodgett, 2017; Doble and Woodgett, 2003; Gao et al., 2013; Xu et al., 2009; Wu and Pan, 2010; Riobó et al., 2006). The large number of GSK3β substrates explains its emblematic function as tumor promoter or tumor suppressor. Those roles have already been extensively summarized in many recent review articles (Sutherland, 2011; McCubrey et al., 2016; Duda et al., 2020; Xie and Wang, 2017).

The most common targets of GSK3β are primed substrates harboring a pre-phosphorylated sequence S/T-X-X-X-S/T(P). Specifically, this provides the binding site for GSK3β, inducing a functional conformation change that assists the target positioning in the active catalytic domain of the kinase (Fig. 1) (ter Haar et al., 2001; Dajani et al., 2003). Hence, the kinase activity of GSK3β leads to either suppression and proteasomal degradation or enhanced activation and protein stabilization of target substrates.

Notably, GSK3β has the unconventional characteristic for a kinase of being normally active in cells under resting conditions. This is mainly correlated with phosphorylation of its tyrosine (Y)216 residue, which induces a conformational change that allows the interaction and phosphorylation of protein targets (Hughes et al., 1993; Kaidanovich-Beilin and Woodgett, 2011). On the other hand, extracellular signals, negatively regulate GSK3β kinase activity *via* N-terminal phosphorylation of the serine (S)9 residue which is required for the maintenance of normal cell homeostasis (Sutherland, 2011; Frame et al., 2001). Crystal structure analysis revealed that phosphorylation of the inhibitory serine-9 residue causes the self-association of the GSK3β N-terminal tail to its substrate binding pocket, thus hampering the interaction with target substrates (Frame et al., 2001; Stamos et al., 2014).

While there are multiple mechanisms that modulate GSK3β activity, they have not been yet completely elucidated due to the highly complex interconnections with several molecular signaling cascades. Interestingly, consistent experimental evidence reported that various regulatory protein kinases such as Akt, cyclic adenosine monophosphate (cAMP)-dependent, protein kinase A (PKA), p70 S6 kinase (p70S6K), p90RSK and Notch3, increase the inhibitory GSK3β serine-9 phosphorylation in response to extracellular signals (Fig. 1) (Kaidanovich-Beilin and Woodgett, 2011; Fang et al., 2000; Foltz et al., 2002). In addition, growth factors such as EGF, PDGF and insulin inhibit GSK3 activity *via* induction of the phosphatidylinositol-3-kinase (PI3K)/MAPK pathway. Other mechanisms that alter the inhibitory phosphorylation status of GSK3β are represented by elevated intracellular cAMP levels mediated by PKA and amino acid deprivation caused by mTOR signaling (Fig. 1) (Krause et al., 2002; Li et al., 2000).

Next to the phosphorylation status of GSK3β, which dynamically oscillates in response to extracellular signals and substrate availability, other varying and controversial mechanisms regulate GSK3β kinase activity such as GSK3β localization and protein-complex formation

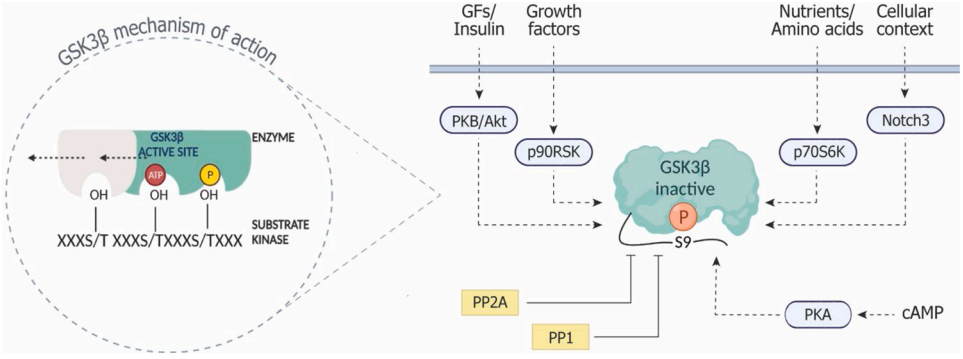


Fig. 1. Mechanism of action of GSK3β with a special focus on mechanisms regulating GSK3β activity by phosphorylation of N-terminal Serine-9. A. GSK3β recognizes a specific amino acid sequence motif S / T-XXX-S / T (P), in which S represents a serine, T a threonine, X a generic amino acid and P indicates the presence of a phosphate group previously bound by another protein kinase which is called kinase primer. The presence of the phosphorylated residue in the recognition sequence allows the substrate to position itself at the active site, thus placing the S/T residue of the target sequence near the kinase site, allowing its phosphorylation. B. Extracellular signals lead to the activation of transduction cascades that result in the phosphorylation of the serine-9 (S9) residue which blocks the target substrate binding and inactivate the kinase activity of GSK3β. Kinase phosphorylating S9 residues are represented with blue ovals and dashed lines. Protein phosphatases PP2A and PP1 restore GSK3β catalytic activity.

(Kaidanovich-Beilin and Woodgett, 2011; Beurel et al., 2015).

Although the exact mechanisms that govern GSK3β trafficking are not fully understood, GSK3β is mainly considered a cytoplasmic protein, with active kinase form more likely found in the nucleus and mitochondria in response to cell cycle stimuli (Bijur and Jope, 2003). GSK3β function in the cytoplasm is primarily related to its recruitment in pre-assembled or signal-induced protein complexes. A classic example is the β-catenin destruction complex in the Wnt signaling cascade among resting conditions, where GSK3β mediates its tumor suppressor action (Wu and Pan, 2010; Komiya and Habas, 2008). Within this complex, GSK3β phosphorylation on Thr41, Ser37 and Ser33 of β-catenin after casein kinase 1 (CK1) priming phosphorylation, results in β-catenin recognition and subsequent ubiquitin-mediated proteasomal degradation, thus modulating the transcriptional activation of target genes (Komiya and Habas, 2008).

Dysregulation of GSK3β has been implicated in diverse pathological entities due to its master function as molecular hub orchestrating the crossroad of multiple essential signals cascades that regulate cell homeostasis, cell survival, differentiation, stemness and epithelial to mesenchymal transition (EMT).

2.2. *The tumor-promoting properties of aberrant GSK3β in pancreatic cancer cells*

Although GSK3β has been recognized to act as a tumor suppressor against several pro-oncogenic molecules and mediators of EMT, aberrant overexpression of GSK3β is implicated in many human malignancies including PDAC (McCubrey et al., 2014) (Fig. 2). Ubiquitous expression and activity of GSK3β have been described to participate in tumor cell survival, apoptosis suppression, cell proliferation and invasion, cancer stemness induction as well as in promotion of chemotherapy resistance (Kockeritz et al., 2006; Ougolkov et al., 2006). Clinical evidence reported that GSK3β-overexpressing PDAC with low Ser9 phosphorylation, inflict a negative prognosis due to sustained tumor promoting signals (Garcea et al., 2007).

The mechanisms leading to tumorigenesis and increased GSK3β in PDAC were investigated by Ding and colleagues. They observed a progressive increase in GSK3β expression in tumor specimens of PDAC patients, correlating with altered oncogenic KRas status (Garcea et al., 2007; Eser et al., 2014; Waters and Channing, 2018; Kazi et al., 2018). Indeed, overexpression of constitutively active Ras isoforms has been registered in approximately 95 % of PDAC patients. Therefore, the subsequent induction of Ras-driven MAPK signaling, in turn, enhances GSK3β expression and alters cancer cell plasticity (Zhang et al., 2011).

In PDAC cell lines, aberrant GSK3β expression and phosphorylation status are also accompanied by a subsequently enhanced nuclear accumulation of active GSK3β, further suggesting the involvement of GSK3β activity in PDAC pathogenesis and progression (Ougolkov et al., 2006). Specifically, deregulated GSK3β expression and activity in PDAC cells result in many pro-survival signals, mainly mediated by NF-kB, JNK, Rb, Notch, TFEB, C-Myc, TP53, WNT/β-catenin signaling pathways (Fig. 3) (Nagini et al., 2019).

Among all, the pro-carcinogenesis role of NF-kB has been extensively described in different cancers, due to its fundamental activity in sustaining tumor cell survival and growth, as well as in modulating cancer cell metabolism and inflammatory microenvironment (Xia et al., 2014; Kaltschmidt et al., 2018). In PDAC, active GSK3β positively regulates NF-kB transcriptional activity at a pathway site, downstream of the IκB kinase complex, thus sustaining NF-kB mediated pro-survival gene expression (Ougolkov et al., 2006; Wilson and Baldwin, 2008; Ougolkov et al., 2005).

Sustained cell survival also appears to be maintained by GSK3β-dependent negative regulation of apoptotic stimuli induced by tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), mainly through promotion of the expression of the pro-survival molecules Bcl-XL, Bcl-2 and Mcl-1. These observations were confirmed by experimental inhibition of GSK3β which resulted in PDAC cell sensitization to TRAIL-induced apoptosis (Zhang et al., 2014; Mamaghani et al., 2012).

The intricate link between GSK3β and the PI3K/PTEN/Akt/mTOR signaling axis may further promote PDAC cell proliferation and tumor progression. AKT, the central node of PI3K transduction cascade regulates GSK3 activity, influencing the inhibitoryS9 phosphorylation (Hermida et al., 2017). Thus, sustained Akt activity and consequent GSK3β inhibitory phosphorylation, lead to increased cyclin D1 and promotes G1/S cell cycle progression (Liang and Slingerland, 2003). However, other studies reported that in pancreatic cells, some pools of GSK3β maintain their functional kinase activity irrespective of AKT activation and consequent inhibition of GSK3β. This evidence further highlights the complex interplay within GSK3 and this mitogenic signaling cascade (Ougolkov et al., 2005).

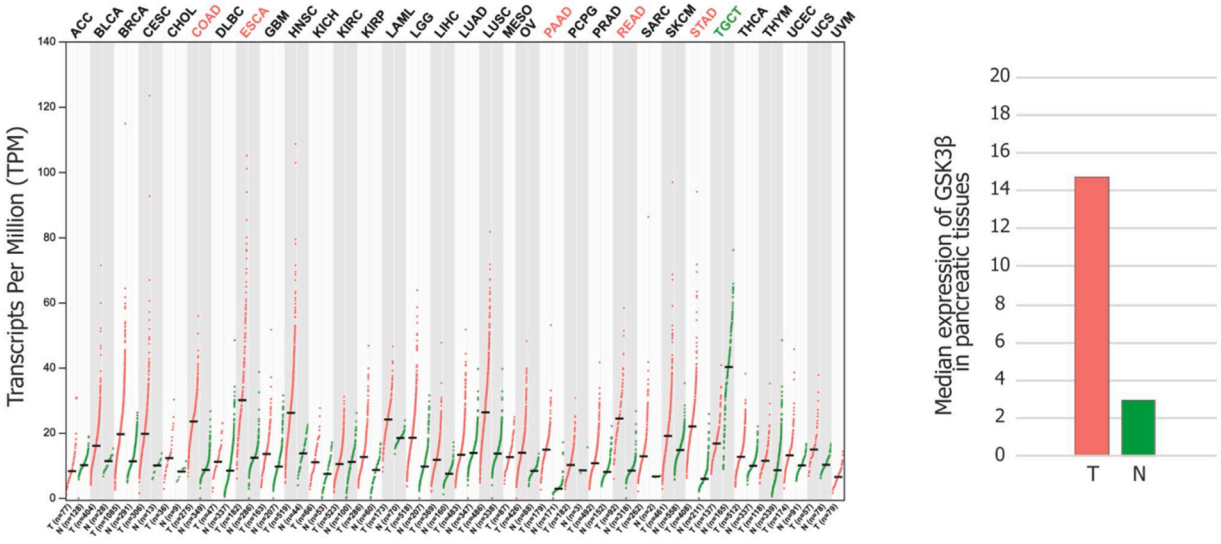


Fig. 2. Studies evaluating GSK3β gene expression levels in tumor samples and paired normal tissues. GSK3β is overexpressed in different tumor types, including pancreatic cancer (PAAD) resulting from the analysis of RNA sequencing expression data of 179 pancreatic tumors and 171 normal pancreatic samples from the TCGA and GTEx projects (http://gepia.cancer-pku.cn/detail.php?gene=GSK3b). Each dot on panel A represents GSK3β expression in tissue samples. Accordingly, the height of bars in panel B represent the median expression of GSK3β in pancreatic tumors (14,97) or pancreatic normal tissues (2,97).

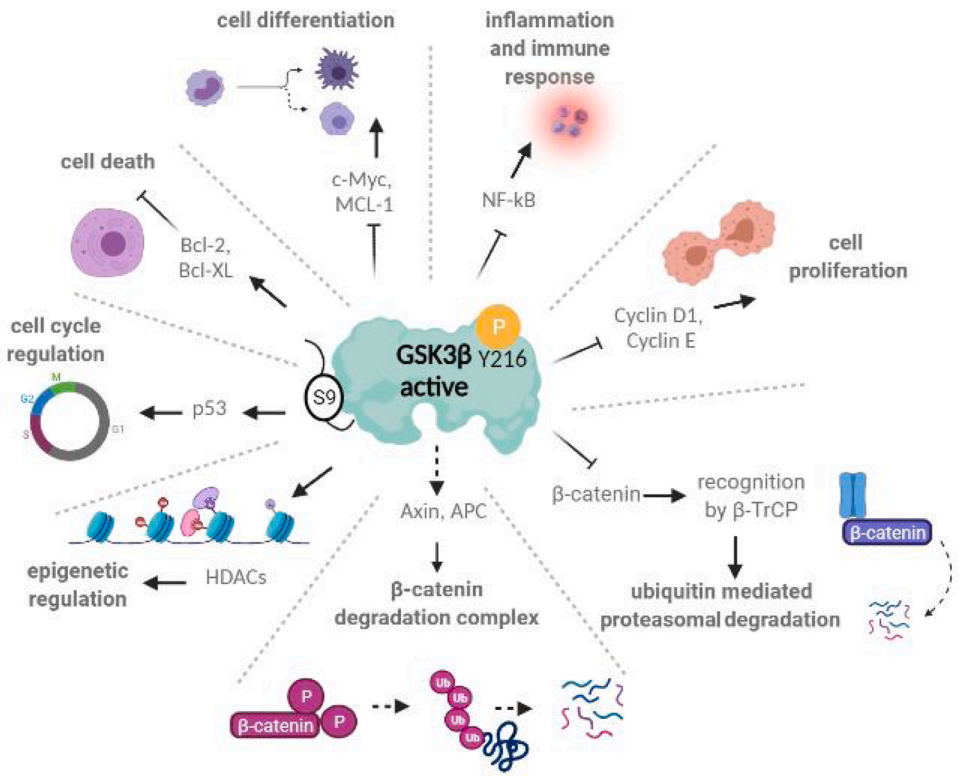


Fig. 3. Multiple roles of GSKβ and its target substrates in key biological processes for cancer cells. GSK3β modulates the activity of many cellular substrates involved in cell cycle progression, cell proliferation and differentiation (p53, c-Myc, MCL-1, Cyclin D1, Cyclin E). GSK3β regulates NF-kB and CREB (not shown) transcription factors affecting inflammatory and immune responses. In the absence of Wnt ligands, the Axin-APC-CK1-GSK3β-β-catenin destruction complex allows GSK3β to phosphorylate β-catenin (residues 41, 37, and 33). This phosphorylation leads to the release of β-catenin from the complex and targets it for proteasomal degradation. GSK3β phosphorylates several histone deacetylases (HDACs) modulating their regulatory epigenetic functions. GSK3β-mediated phosphorylation of some molecules of the anti-apoptotic Bcl-2 family results in their stabilization and increased anti-apoptotic effects.

Additionally, recent findings showed that GSK3 cooperates with mTOR to regulate the activity of p70 ribosomal protein S6 kinase 1 (S6K1), which is a pivotal regulator of extracellular signals supporting cell growth (Shin et al., 2011). Other outstanding mechanisms sustaining cancer cell survival and proliferation involve modulation of c-Myc signaling, Hedgehog (Hh) signaling and STAT3 cascade, but further research is required in the context of PDAC (Baumgart et al., 2016; Singh et al., 1995).

Moreover, the WNT/β-catenin signaling pathway plays an important role in the modulation of apoptosis, differentiation, invasion and epithelial to mesenchymal transition, all critical hallmarks for cancer metastasis (Doble and Woodgett, 2007). GSK3β is a well-established regulator of β-catenin subcellular localization and deregulated GSK3β activity may severely impact the tumor-suppressive and tumor-promoting roles of the WNT/β-catenin signaling cascade (Domoto et al., 2016). Consistently, pharmacologic inhibition of GSK3β was shown to upregulate β-catenin and c-Myc levels, as well as suppress tumor growth in KRas-mutant PDAC and non-small lung cancer models (Kazi et al., 2018).

Interestingly, it has been recently observed that GSK3β plays a key role in modulating cell cycle progression at different regulatory levels. In pancreatic cancer models, GSK3β was observed to directly support the phosphorylation status of many cell cycle modulators such as cyclin D1, p53 and various transcription factors (Kitano et al., 2013; McCubrey et al., 2016). The growth promoting function of GSK3β in this tumor type is supported by both *in vitro* and in tumor xenograft experiments *in vivo*: specifically, pharmacological inhibition of GSK3β activity was reported to promote apoptosis by suppressing Cyclin D1 expression, as well as impairing the transcriptional activity of E2F transcription factor and consequent phosphorylation of the Rb protein (Kitano et al., 2013). Furthermore, Yoshino and colleagues observed that apoptosis-resistant PDAC cells treated with GSK3β inhibitors, exhibited biodynamic cell mechanisms typical of mitotic catastrophe (Yoshino and Ishioka, 2015). This study provided the first proof of fundamental role of GSK3β in controlling mitotic processes in PDAC cells. Similar observations were also recently made in colorectal cancer cells (Yoshino and Ishioka, 2015; Dewi et al., 2018).

Overall, within the complexity of the GSK3β signaling cascades in pancreatic tumorigenesis and tumor progression, cumulative evidence renders GSK3β a promising therapeutic target in order to improve the survival of PDAC patient and enhance the therapeutic responses.

2.3. *Chemoresistance in advanced or metastatic pancreatic cancer*

Surgical resection of PDAC remains the curative treatment choice for achieving long-term survival. However, more than 85 % of PDAC patients are diagnosed with advanced-stage or metastatic disease which is not generally amenable to pancreatectomy (Meijer et al., 2020).

The standard-of-care intervention in those tumors with aggressive-stages mainly include chemotherapeutic agents, such as gemcitabine, 5-fluorouracil, oxaliplatin, irinotecan and nab-paclitaxel (El Hassouni et al., 2019). However, after a good initial response of sensitive tumors, overt chemoresistance eventually develops within weeks, thus severely limiting the effectiveness of those therapeutic interventions (Zeng et al., 2019).

Particularly, PDAC cells showed stronger intrinsic or acquired insensitivity to gemcitabine. Recent phase III clinical trials reported superior overall survival in patients with advanced or metastatic PDAC receiving nab-paclitaxel plus gemcitabine or FOLFIRINOX compared to gemcitabine alone (Caparello et al., 2016). However, although these treatments achieved a longer survival rate than gemcitabine use alone, combinations of chemotherapeutic agents generally show higher frequency of toxicity and patients still become resistant after short times (Pusceddu et al., 2019).

Therefore, gaining insights into tumor intrinsic or acquired strategies responsible for chemotherapy resistance are of urgent need in order to develop novel targeted therapeutic approaches that improve patient overall survival, lower toxicity profile and overcome chemoresistance in advanced or metastatic PDAC (Zeng et al., 2019).

2.3.1. *GSK3 β and chemoresistance*

Even though the underlying mechanisms which trigger

chemoresistance remain controversial, defective pharmacodynamics of biochemical mechanisms, together with perturbations on several cellular signaling cascades, were found in gemcitabine-resistant PDAC cells. These mainly involve nucleoside transport and metabolism, reactivation of EMT and developmental pathways, such as WNT/β-catenin, Hh and Notch, and growth factor signaling (El Hassouni et al., 2019; Randazzo et al., 2020; Saiki et al., 2012; Ireland et al., 2016; Shukla et al., 2017).

As previously described, mounting evidences report that aberrant activation of NF-kB plays a crucial role in uncontrolled cell proliferation, tumorigenesis, metastasis, angiogenesis, inflammation and chemotherapy resistance in PDAC (Arlt et al., 2003; Okamoto et al., 2007; Liptay et al., 2003; Holcomb et al., 2008; Mamaghani et al., 2009). Specifically, a strong correlation was observed between basal level of NF-kB activity and gemcitabine resistance. Indeed, in resistant PDAC cell lines (PancTu-1, Capan-1 and BxPc-3 cells) a strong activation of NF-kB was detected when compared to sensitive T3M4 and PT45-P1 PDAC cell lines (Arlt et al., 2003).

NF-kB contribution to chemoresistance was further confirmed by pharmacologic inhibition and by targeting IkBα super-repressor or GSK3β, which resulted in increased sensitivity to gemcitabine in non-responsive PDAC cell lines (Arlt et al., 2003). Mechanistically, GSK3β has been shown to positively regulate NF-kB maintaining high NF-kB activity, thus evidencing GSK3β contribution to chemoresistance onset in PDAC (Ougolkov et al., 2006, 2005; Walz et al., 2017).

PI3K/Akt-mediated signal transduction is another important anti-apoptotic signaling cascade that has been related to chemoresistance of PDAC. Akt is the primary mediator of PI3k-initiated signaling cascade and is specifically involved in the phosphorylation and subsequent inactivation of pro-apoptotic molecules such as Bad, IKb (Ikk β) kinase, caspase-9, the forehead family of transcription factors (FKHR/AFX/FOX), CREB, Raf, p21, as well as GSK3 (Massihnia et al., 2017). A recent study reported that activation of Akt and inhibition of GSK3β through Akt-mediated Serine 9 phosphorylation resulted in the upregulation of Snail1 expression through increased protein stability, promoting EMT-like phenotype and gemcitabine tolerance (Namba et al., 2015).

In order to define the stepwise processes triggering gemcitabine resistance in the clinic, Uehara and colleagues developed a gemcitabine-resistant systemic model derived from the gemcitabine-sensitive human PDAC cell line BxPC-3 (Uehara et al., 2020). Through their work, they demonstrated that GSK3β facilitates the capacity of PDAC to tolerate chemotherapy by interfering with Rb protein function and E2 transcription factor (E2F)1-mediated transcription. Specifically, gemcitabine-resistant clones were characterized by high expression of ribonucleotide reductase M1 (RRM1), a well-known transcriptional target of the pro-oncogenic E2F1 (Uehara et al., 2020; Yoneyama et al., 2015). Additionally, pharmacological inhibition of GSK3β was proved to re-sensitize resistant cells to gemcitabine by restoring the functional Rb-mediated regulation of E2F1, attenuating E2F1 transcriptional activity and consequently decreasing RRM1 expression (Uehara et al., 2020; Kitano et al., 2013).

In summary, GSK3β seems to be involved in many different processes that promote resistance of PDAC cells to gemcitabine and other drugs by sustaining the invasive capacity and stemness phenotype of pancreatic malignant cells.

2.4. Targeting GSK3β in pancreatic cancer

GSK3β regulation of several molecular promoters of neoplastic transformation, together with the shorter survival of PDAC patients harboring high GSK3β expression, strongly sustain the hypothesis of GSK3β central involvement in PDAC onset and progression (McCubrey et al., 2016). Thus, accumulating evidence on GSK3β functions has proven the rationale for the clinical development of novel therapeutic strategies targeting GSK3β in advanced PDAC (Cormier and Woodgett, 2017; Baumgart et al., 2016; Walz et al., 2017; Hoeflich et al., 2000).

The therapeutic and antitumor effects achieved by GSK3β inhibition have been described in different cancer types, as reviewed recently by Domoto and colleagues (Domoto et al., 2020). Notably, some evidence reveals that GSK3β inhibition leads to induction of apoptosis in PDAC cells, whereas normal pancreatic epithelial cells seem to be protected from the inhibitory effects associated with the targeted treatment (Marchand et al., 2012). This might find an explanation in the intrinsic biological nature of PDAC cells which present high levels of active GSK3β aberrantly accumulated in the nucleus (Ougolkov et al., 2006; Walz et al., 2017). Thus, PDAC may become more sensitive to the proteasome-dependent GSK3β loss from the nucleus that is induced by the treatment with GSK3 inhibitors (Marchand et al., 2012, 2015).

Inhibition of GSK3β may therefore be a promising precision medicine strategy in PDAC treatment (Domoto et al., 2020; Baudino, 2015). Through cancer cell death promoting effects, this approach may restrict tumor recurrence and metastasis as well as spare harmful consequences on healthy cells and tissues, frequently associated with conventional cytotoxic therapies.

2.4.1. GSK3 β inhibitors

Multiple GSK3β inhibitors have been developed and many others are now under investigation (Table 1), as previously reviewed by Saraswati AP et al., and Eldar-Finkelman H et al. (Saraswati et al., 2018; Eldar-Finkelman and Martinez, 2011). In the early 1980s, the cation lithium was the first GSK3β inhibitor being described and then approved by the Food and Drug Administration (FDA) for the treatment of human bipolar depression (Johnson and Amdisen, 1983; Freland and Beaulieu, 2012; Bowden, 2000).

Studies on the mechanism of action of lithium showed that it disrupts the catalytic function of GSK3β by competing for the binding of magnesium (Mg^{2+}) cofactor, but not for ATP or the substrate (Ryves and Harwood, 2001; Phiel and Klein, 2001; Pasquali et al., 2010). Additionally, lithium indirectly increased the N-terminal inhibitory phosphorylation of GSK3β either by enhancing the activity of Akt, through the regulation/dissociation of the Akt:β-arrestin 2 (βArr2): protein phosphatase 2A (PP2A) signaling complex, or by blocking the dephosphorylation of PKB (Pasquali et al., 2010; Zhang et al., 2003; Mora et al., 2002; De Sarno et al., 2002).

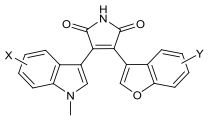
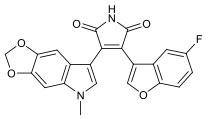
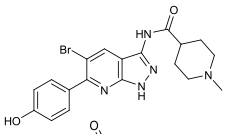
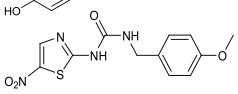
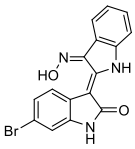
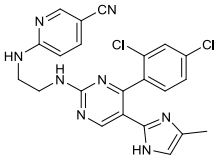
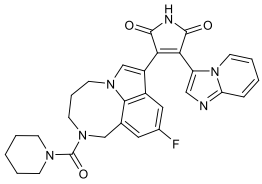
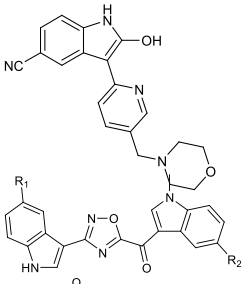
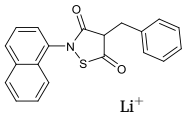
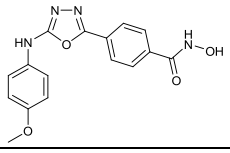
Experimental studies on PDAC cells reported reduced tumorigenic potential and cell proliferation, as well as increased apoptosis among lithium treatment (Peng et al., 2013). This outcome was associated with the enhanced ubiquitin-dependent proteasome degradation of the glioma-associated oncogene-1 (GLI1), a crucial downstream component of the Hh signaling pathway, following GSK3β inhibition (Peng et al., 2013; Zhu and Lo, 2010).

The ATP-binding pocket is an essential site for the catalytic action of GSK3β; thus, various GSK3β inhibitors competing with ATP molecules have been developed in order to block the kinase activation. However, one has to note that, whereas those agents might selectively target GSK3β, they can also exert inhibitory effects towards cyclin-dependent kinases (CDKs) since some of them including CDK2, share very similar ATP-binding pockets with GSK3β (Vulpetti et al., 2005). Therefore, the dual inhibitory function of ATP-competitive GSK3β inhibitors may directly impact cell cycle regulation and enhance the antiproliferative outcome.

Gaisina and colleagues designed a preliminary library of benzofuran-3-yl-(indol-3-yl) maleimides, including some selective and potent ATP competitive inhibitors of GSK3β. *In vitro* evaluation of the therapeutic potency of these maleimides showed that compounds **1a-e** exhibited promising antiproliferative effects against a panel of PDAC cell lines (MiaPaCa-2, HupT3 and BXPC2) (Table 1) (Gaisina et al., 2009). Among all, treatment with compounds **1a** and **1e** resulted in pronounced inhibition of GSK3β activity, which correlated with reduced NF-kB-mediated expression of the antiapoptotic X-linked inhibitor of apoptosis protein (XIAP).

Recently, **1e** (9-ING-41), maleimide-based ATP-competitive GSK3β

Table 1
GSK3β Inhibitors in pancreatic cancer preclinical and clinical studies.

Category	Inhibitor	Structure	Pharmacologic activity	GSK3β inhibitory effects	Ref/Clinical trial
ATP competitive	benzofuran-3-yl-(indol-3-yl) maleimides		Potent ATP competitor	Apoptosis induction, reduced NF-kB-mediated expression of XIAP	Gaisina IN et al., 2009
	9-ING-41		Potent ATP competitor	Cell cycle arrest, reduced expression of anti-apoptotic molecules Bcl-2 and XIAP	Ding L et al., 2017 NCT03678883, 1801 pase 1/2 study
	SB-732881-H		Potent ATP competitor	Apoptosis induction, increased expression of the Bcl-2 protein family	Marchand B et al., 2012
	AR-A014418		Potent ATP competitor	Lowers cytoplasmic β-catenin levels and abrogates NF-kB transcriptional activation	Bhat et al. (2003) Mamaghani S et al., 2009
	BIO		Reduced inhibitory S9 phosphorylation	Enhanced apoptosis via JNK-dependent mechanism	Meijer L. et al., 2003 Kazi A et al., 2018
	CHIR99021		Potent ATP competitor	Apoptosis induction	Marchand B et al., 2012 NCT01287520, 2018
	LY2090314		Increased inhibitory phosphorylation	Suppressed pro-survival signals	NCT01632306, 2019
	AZD-1080		Potent ATP competitor	Reduced cell cycle progression related genes	Kazi A et al., 2018
	Library of synthetic topsentin analogs		Docked in the ATP binding site	Pro-apoptotic signals induction, reduced expression of EMT markers	Carbone D et al., 2021
Non-ATP competitive	Tideglusib		Binding site not yet defined	Cell cycle arrest, impaired phosphorylation of β-catenin and c-Myc	Kazi A et al., 2018 ; Domínguez JM et al., 2012
	Lithium	Li^+	Compete for Mg^{2+} cofactor binding	Suppressed hedgehog signaling pathway	Peng Z et al., 2013
Dual inhibitor	Metavert		Inhibition of GSK3β and HDAC-2	Cell cycle arrest, reduced expression of EMT markers	Edderkaoui M et al., 2018

inhibitor, showed cytostatic effects in PDAC models and is now under investigation in a phase I/II clinical trial (NCT03678883) in patients with advanced solid tumors (Ding and Billadeau, 2020; NCT03678883, 2021; Carneiro et al., 2020). Even if available results reported a good 9-ING-41 monotherapy tolerance and antitumor efficiency more promising therapeutic effects were obtained under combination chemotherapeutic regimens in patients with refractory PDAC (Carneiro et al., 2020; Ding et al., 2019).

Increased sensitivity to chemotherapy or chemoresistance regression upon treatment with 9-ING-41 have been newly discovered to be related to GSK3β regulation of the gemcitabine-induced TopBP1/ATR/Chk1 DNA damage response pathway, as will be discussed later (Ding et al., 2019).

SB-732881-H (SB), a dual inhibitor of both GSK3 isoforms, selectively suppressed the viability of mutant KRas-dependent tumor cells (Kazi et al., 2018; Fleming et al., 2005). *In vitro* studies on human PDAC cellss MiaPaCa2 harboring aberrant KRas, revealed high sensitivity to SB treatment (IC_{50} of 0.4 μM), resulting in caspase-3 activation and induction of PARP cleavage (Kazi et al., 2018; Demarchi et al., 2003). Furthermore, additional reproducible data suggested that the antitumor effect of SB on KRas-mutant PDAC is supported by apoptosis induction in a c-Myc- and β-catenin-dependent manner (Kazi et al., 2018). In contrast, pharmacologic activity of SB did not affect the viability of non-malignant pancreatic epithelial cells. Overall, these findings are consistent with the pro-survival function of GSK3 in PDAC and further support the assumption that mutant KRas tumors are dependent on GSK3α/β signalling for cancer cell survival and tumor growth (Kazi et al., 2018; Saiki et al., 2012; Fleming et al., 2005; Demarchi et al., 2003; Bang et al., 2013).

AR-A014418, another dual GSK3 inhibitor, induced a strong dose-dependent growth reduction in various pancreatic tumor models (Bhat et al., 2003; Kunnimalaiyaan et al., 2015). By selectively competing for the ATP-binding pocket of GSK3β, AR-A014418 lowered the cytoplasmic β-catenin levels and abrogated NF-kB activation, thus reducing the expression of NF-kB target genes cyclin D1, XIAP and Bcl-XL (Mamaghani et al., 2009; Bhat et al., 2003). Moreover, a recent *in vitro* study reported that AR-A014418 inhibition of GSK3α phosphorylation decreased the expression of Notch pathway members, thus attenuating tumor cell survival (Kunnimalaiyaan et al., 2015).

6-bromoindirubin-3′-oxime (BIO), a synthetic analog of natural indirubins, has been studied in the context of drug resistance in many different cancer types for its indirubin-related property to concomitantly inhibit CDKs and GSK3β (Zhang et al., 2017; Liu et al., 2017; Li et al., 2018;), *via* interaction with the ATP-binding pocket of both kinases (Meijer et al., 2003).

Studies on beneficial effects of BIO have been conducted in the context of anti-aging properties, where treatment with 6-bromoindirubin-3′-oxime reported ameliorated lipid metabolism and positive modulation of autophagy, inflammation and oxidative stress (Guo et al., 2019).

In PDAC cells, treatment with BIO directly inhibited GSK3β *via* suppression of Tyr216 phosphorylation and enhanced apoptosis through JNK-dependent mechanisms (Marchand et al., 2015). However, Marchand and colleagues observed that BIO-GSK3β inhibition augmented the activation of the autophagy/lysosomal network which was elicited through enhanced nuclear localization of the transcription factor EB (TFEB), a master regulator of autophagy and lysosomal biogenesis (Marchand et al., 2015; Zhitomirsky and Assaraf, 2016; Zhitomirsky et al., 2018; Zhitomirsky and Assaraf, 2015). Although the autophagy effects on cancer cells are not well defined, it has been reported that sustained autophagy in PDAC cells elicits cell proliferation by overcoming death signals and favoring oxidative phosphorylation (Yang and Kimmelman, 2011). This metabolic switch provides a proper bioenergetic metabolism and pivotal survival signals to malignant cells under restrictive growth conditions, thus supporting cancer cell growth and tumor progression. Overall, these undesired modulatory effects exerted by BIO, hampered its progression into clinical trials.

Comparably to BIO, the aminopyrimidine derived GSK3 inhibitor CHIR99021 exhibited both apoptosis induction and concomitant increased autophagic response *via* LC3B II expression in PDAC cell models (Tran and Zheng, 2017). Pharmacologic depletion of vacuolar H^+ ATPase with bafilomycin A1, prevented autophagy by disrupting lysosomal acidification, thus forcing PDAC cells to preferentially respond to the death signals mediated by CHIR99021. Indeed, this study supported the hypothesis of addressing autophagy induction as a promising mechanism of escape to the antiproliferative effects of GSK3 inhibitors in the setting of PDAC treatment.

Conversely, LY2090314, an ATP-competitive and highly selective GSK3 inhibitor, is currently under clinical evaluation for cancer treatment (clinical trials: NCT01632306 Phase I/II, NCT01287520 Phase I). *In vitro* and *in vivo* studies showed that treatment with LY2090314 in PDAC models increased the inhibitory phosphorylation of GSK3 and significantly suppressed the expression and pro-survival activity of TGFβ-activated kinase 1 (TAK1), a crucial mediator of cellular signals that sustain PDAC aggressiveness and chemoresistance (Bang et al., 2013; Melisi et al., 2011; Giovannetti et al., 2014). Moreover, Santoro and co-workers demonstrated that reduced TAK1 expression induced by pharmacologic inhibition of GSK3 impacts the YAP/TAZ functions in PDAC cells, thus affecting their contribution to the progression and drug insensitivity of this malignancy (Santoro et al., 2020; Lin et al., 2015).

The GSK3 inhibitors AZD-1080 and Tideglusib were first designed and tested for the treatment of Alzheimer's disease (AD). Notably, they are currently under investigation in the context of cancer therapy (Lovestone et al., 2015). Ovarian carcinoma cells exposed to AZD-1080 showed a significant downregulation of GSK3β, as well as cell cycle progression related genes at both the transcript and protein levels (Chen et al., 2016). The high selectivity and remarkable suppression of cancer cell proliferation following AZD-1080 treatment has been recently confirmed in PDAC cell lines.

As BIO and AZD-1080, Tideglusib showed selective inhibitory activity on GSK3β, impairing the phosphorylation of many GSK3 targets, including β-catenin and c-Myc in refractory PDAC cells and models harboring mutated KRas (Kazi et al., 2018; Domínguez et al., 2012). Mechanistically, Tideglusib differs from the ATP-competitive hallmark of AZD-1080, eliciting an irreversible inhibition of GSK3β *via* a non-competitive mode, although the exact binding site of this molecule has not been elucidated yet (Domínguez et al., 2012).

Interestingly, Edderkaoui and colleagues observed that even though the treatment with Tideglusib in PDAC cells (HPDE6, Bx-PC3, MIA PaCa-2 and HPAF-II) promoted the expression of the EMT marker vimentin, the combinatorial inhibition of histone deacetylases (HDAC) class I-II and GSK3β reduced cancer cell survival and the levels of EMT markers (Edderkaoui et al., 2018).

Based on this observational study, they developed Metavert, a novel synthetic molecule designed by combining Tideglusib and suberoylanilide hydroxamic acid (SAHA) active pharmacophores. SAHA (Vorinostat) is an FDA approved inhibitor of HDAC classI-II currently used for the treatment of cutaneous T cell lymphoma. By inhibiting both GSK3β and HDAC-2, Metavert synergistically impaired *in vitro* PDAC cell proliferation and prevented drug resistance as well as the expression of migration-, EMT- and stemness–associated markers. Furthermore, it significantly reduced tumor cell growth, preventing metastasis and improving overall survival in aggressive PDAC mouse models (Edderkaoui et al., 2018)

Recently, a new library of synthetic topsentin analogs with a central replaced 1,2,4-oxadiazole ring reported promising 50 % growth inhibition values against a panel of different human cancer cell lines [49]. Specifically, five of these newly synthetized agents (**2a-e**) effectively reduced cancer cell viability in PDAC cells (Panc-1, SUIT-2 and Capan-1), with compound **2a** displaying the highest cytotoxic activity (IC_{50} range 0.40–1.19 μM) (Supplemental Table 1) (Carbone et al., 2021). Compounds **2a** and **2e** significantly reduce GSK3β phosphorylation in Panc-1 cells, potentially impacting cancer cell survival and tumor progression. Overall, *in vitro* studies revealed that the antiproliferative effects of these novel topsentin derivatives correlated with apoptosis induction, reduced cell migration and expression of the EMT markers SNAIL-2 and metalloproteinase-9, thus paving the way for new promising studies for the treatment of PDAC with GSK3 inhibitors (Carbone et al., 2021).

2.4.2. Combined therapy with GSK-3β inhibitors

Limited second line therapy approaches are currently available for the management of refractory PDAC. Multiple studies in cancer models reported that certain GSK3β inhibitors enhance tumor sensitivity to chemotherapeutic agents (Abrams et al., 2021; Miyashita et al., 2009; Shimasaki et al., 2012). Therefore, in the past decade various GSK3β inhibitors have been experimentally and clinically tested in the context of refractory and advanced metastatic PDAC with the purpose of developing effective therapeutic strategies that could prevent or overcome drug resistance, while lowering chemotherapy-associated untoward toxicity (Table 2). *In vitro* and *in vivo* research showed that, as gemcitabine cytotoxicity is dependent on cell cycle regulatory processes, pharmacologic inhibition of GSK3β can prevent DNA damage repair inflicted by gemcitabine and induced apoptosis in chemoresistant PDAC cells (Ding et al., 2019; Shimasaki et al., 2012). For example, 9-ING-41 ameliorates the antitumor effects of gemcitabine through modulating the ATR-Chk1 DNA damage response (Ding et al., 2019). In fact, observational studies reported that pharmacologic inhibition of GSK3β in PDAC cells triggers topoisomerase IIβ binding protein (TopBP1) degradation and impairs ATR activation, consequently reducing gemcitabine-mediated Chk1 phosphorylation (Ding et al., 2019). Additionally, in contrast to results obtained by Mamaghani and colleagues, novel experimental evidence reported that AR-A014418 not only suppressed proliferation of PDAC cells and impaired tumor growth, but also synergistically sensitized tumor cells to gemcitabine treatment. Indeed, transcriptome profiling revealed that inhibition of GSK3β counteracts the gemcitabine-induced expression of DNA repair, cell death and autophagy–related genes, such as the tumor protein 53-induced nuclear protein 1 (TP53INP1) (Mamaghani et al., 2009; Shimasaki et al., 2012).

Further research revealed that LY2090314 synergistically interacts with clinically relevant chemotherapeutic agents gemcitabine, oxaliplatin, nab-paclitaxel and SN-38, the active metabolite of irinotecan (Santoro et al., 2020; Lin et al., 2015), by modulating the intrinsic chemoresistance of PDAC cells. Interestingly, while treatment with drug combinations decreased PDAC cell viability, mice treated with LY2090314 and nab-paclitaxel exerted improved overall survival with reduced cytotoxic effects on non-malignant pancreatic cells (Santoro et al., 2020; Zamek-Gliszczynski et al., 2013).

Moreover, the pioneer GSK3β inhibitor lithium has been observed to synergistically improve the antitumor effect of gemcitabine mainly by perturbing the Hh-GLI cascade and enhancing the proteasome-degradation of GLI (Peng et al., 2013). Next to that, Elmaci and Altinoz suggested that the triple-agent regimen comprising already-in-use drugs metformin, pioglitazone and lithium may synergistically target cancer cell metabolism by activating AMPK and PPAR-γ and perturbing GSK3β, respectively (Elmaci and Altinoz, 2016). In *in vitro* as well as in animal model studies, this triple drug combination increased the intrinsic sensitivity of PDAC cells to apoptosis, potentially providing a novel beneficial adjuvant therapy for refractory PDAC.

Furthermore, Metavert, the dual GSK3β/HDAC inhibitor, when used in combination with irradiation and chemotherapeutic agents paclitaxel or gemcitabine, it reduced tumor growth compared to monotherapy and significantly prolonged the survival rate of mice harboring drug-resistant PDAC (Edderkaoui et al., 2018).

Overall, the combination of GSK3β inhibitors and other chemotherapeutic agents appears to synergistically reduce tumor cell growth and increase survival in different models, revealing encouraging therapeutic effects towards the overcoming of chemoresistance in refractory PDAC and paving the way for future clinical studies.

2.4.3. Clinical studies on drug combination regimens including GSK-3β inhibitors

At present, different drug combinations have been explored in preclinical and early-phase clinical studies in order to meet the urgent demand for efficient strategies in the therapeutic management of advanced and metastatic PDAC. However, none of them has already been approved.

Cumulative preclinical evidence provided the rationale to clinically test 9-ING-41 (phase I/II, NCT03678883) in combination with the standard chemotherapeutic agents gemcitabine, nab-paclitaxel, carboplatin, paclitaxel, doxorubicin, lomustine or irinotecan in patients with advanced or refractory solid tumors, including PDAC (NCT03678883, 2021; Carneiro et al., 2020). Furthermore, the promising preclinical outcome of LY2090314 and platinum combination in xenograft models have prompted the clinical evaluation of LY2090314 plus carboplatin and pemetrexed in patients with advanced or metastatic cancer (phase I, NCT01287520) (NCT01287520, 2018). Although establishing the efficiency of LY2090314 combined with carboplatin and pemetrexed requires further interventional confirmations, LY2090314 safety profile, pharmacokinetic parameters and optimal drug doses were established (NCT01287520, 2018; Gray et al., 2015). A parallel study conducted on patients with acute leukemia showed that LY2090314 was well tolerated and reported good antitumor activity when combined with chemotherapeutics, while minimal clinical benefits were observed if administered as monotherapy (Gray et al., 2015; Rizzieri et al., 2016). Additionally, phase I/II trial (NCT01632306, 2019) assessing the combination regimen of LY2090314 and different chemotherapies (FOLFOX, gemcitabine and nab-paclitaxel) in patients with metastatic PDAC was recently terminated due to slow enrollment procedure (NCT01632306, 2019).

Currently, combination treatment with lithium, cimetidine, olanzapine and valproate regimen (CLOVA cocktail) is under clinical investigation with simultaneous usage of gemcitabine in advanced PDAC patients (UMIN000005095), but no data are available yet.

Various phase II clinical trials on diverse pathologies are actually evaluating possible therapeutic regimens with the GSK3β inhibitor tideglusib (NCT01350362, NCT02586935, NCT02858908), providing potential curative possibilities to be tested in the near future also in

Table 2
Drug combination: with GSK3β inhibitors in PDAC.

Drug Combinations with GSK3β inhibitors in PDAC				
Treatment	GSK3βi effect	Pharmacological interaction	Tumor stage	Ref/Clinical trial ID
AR-A014418 and Gemcitabine	Impaired DNA repair gene regulation and expression. Inhibition of Notch1 expression.	Synergistic	Preclinical models	Kunnimalaiyaan et al., 2015
9-ING-41 and Gemcitabine	Modulation of ATR-Chk1 DNA damage response	Synergistic	Refractory	NCT03678883 Ding et al., 2019
LY2090314 and Gemcitabine, FOLFOX or Gemcitabine + Nab-paclitaxel	CDK-dependent RRM1/2 downregulation and increased DNA damage	Synergistic	Advanced or metastatic	NCT01632306, 2019 Phase I/II NCT01287520, 2018
Metavert and Gemcitabine	Impaired metabolic profile, cell migration capability and cancer stemness. Altered tumor microenvironment.	Synergistic	Locally advanced	Edderkaoui et al., 2018
Lithium and Gemcitabine	Impaired Hh-GLI signaling	Additive	Preclinical models	Peng et al., 2013

patients with PDAC (Lovestone et al., 2015; Horrigan et al., 2020). Nevertheless, it is important to consider that currently available clinical data provide limited information regarding the overall administration-related adverse events of these combination therapeutic regimens. Indeed, the complexity of cellular GSK3β interconnections, the small number of ongoing clinical trial and the fact that none of the GSK3β inhibitors has been approved for clinical use to date, excluding lithium, further complicate the prediction of beneficial or adverse effects in patients. Therefore, additional studies are required before proposing GSK3β inhibitors-based interventions as therapeutic alternatives in the clinical management of advanced and metastatic PDAC.

2.5. Resistance to GSK-3 inhibitors

Experimental studies revealed that various gene programs are activated upon GSK3β inhibition, mainly involving metabolic reprogramming, compensatory pro-survival signaling cascades, hyper-activation of NF-kB and WNT signaling, as well as increased autophagy/lysosomal network activity (Ougolkov et al., 2005; Marchand et al., 2015; Seino et al., 2018; Webster et al., 2000; Sun et al., 2016; Bruton et al., 2020).

Despite the general consensus regarding the antineoplastic activity of GSK3β inhibitors, a more comprehensive analysis on the downstream effects of GSK3β inhibition uncovered the potential induction of escape signals mediated by increased autophagic response (Marchand et al., 2015). Although PDAC cells harbor elevated levels of basal autophagy, whether autophagy displays a tumor suppressor role or a potential resistance mechanism to anticancer therapy, remains elusive (Yang et al., 2011; Galluzzi et al., 2015).

A recent study demonstrated that pharmacologic inhibition of GSK3β in PDAC cells enhanced the transcriptional activity of TFEB, thus positively modulating the autophagic flux (Marchand et al., 2015). Moreover, experimental inhibition of the autophagy cascade with bafilomycin A1 and/or CHIR99021 ameliorated the sensitivity of human PDAC cells to apoptosis triggered upon concomitant treatment with GSK3 inhibitor (Marchand et al., 2015). Similar outcomes were observed in prostate and bladder cancer cells, further sustaining the hypothesis that autophagy exerts a potential mechanism of resistance to GSK3β inhibition and that a combination drug treatment targeting both GSK3β and the autophagy/lysosomal network might prevent this issue (Marchand et al., 2015; Kuroki et al., 2019). Furthermore, autonomous production of stromal WNT ligands, sustaining constitutive Wnt signaling, as well as hyper-activated NF-kB transcriptional activity, have been described in various subsets of PDAC cells (Ougolkov et al., 2005; Seino et al., 2018; Webster et al., 2000; Bruton et al., 2020). Therefore, in light of the molecular interconnections between GSK3β and these signaling pathways, it would not be surprising if these processes might constitute intrinsic mechanisms of resistance to GSK3β inhibition in pancreatic tumor cells.

In this regard, several analyses distinguished two PDAC subtypes, the classical and squamous lineages, which are characterized by distinct transcriptomic and proteomic profiles, as well as prognosis (Collisson et al., 2019; Bailey et al., 2016; Le Large et al., 2020). Specifically, while the classical subtype expresses differentiated endoderm cell markers and mostly experiences favorable clinical outcome, the squamous phenotype harbors altered epigenetic landscape, affecting the expression of duct cell markers and leading to a rapid metabolic reprogramming often leading to a worse prognosis (Bailey et al., 2016; Lomberk et al., 2018; Le Large et al., 2017). This was corroborated by Brunton and colleagues, who reported that a subset of squamous pancreatic cell lines rapidly acquired drug resistance upon treatment with GSK3β inhibitors (Brunton et al., 2020). Specifically, these cancer cells encountered a metabolic adaptation under persistent suppression of glycolysis mediated by pharmacologic inhibition of GSK3β. Furthermore, this was accompanied by an increased dependency on autophagy and activation of a unique gene transcription program resulting in the self-production of WNT ligands, and ultimately leading to drug resistance.

Overall, sustained autophagy flux and activation of compensatory cascades appear to rapidly induce acquired resistance to GSK3β inhibition in PDAC cells. Therefore, further studies are warranted to unravel the complexity of autophagy: potential therapeutic applications in PDAC (Gomez Mellado et al., 2015). However, in-depth analysis of unique chromatin landscape signatures and mutation profiles of PDAC cells might improve our understanding of the dynamics of emergence of drug resistance mechanisms, thus supporting the design of more effective therapeutic approaches.

2.6. Tumor chromatin profiling may predict patients with pancreatic tumors sensitive to GSK3B-targeted therapy

The lack of defined biomarkers and the high disease heterogeneity characterizing PDAC are representative of the difficulties in predicting and determining which patients might respond to targeted therapies.

To date observational data highlighted the link between mutant KRas PDAC and GSK3β overexpression (Zhang et al., 2011; Fleming et al., 2015; Fitzgerald et al., 2015). In fact, oncogenic KRas signaling, positively regulates GSK3β expression and activity, thus favoring cell proliferation, survival and tumor dedifferentiation. Pharmacologic inhibition of GSK3β in KRas-dependent tumors was found to impair cancer cell growth and induce apoptosis, partly mediated by c-Myc- and β-catenin-dependent mechanisms (Kazi et al., 2018; Kim et al., 2000). However, the intrinsic plasticity and aggressiveness of KRas-dependent tumors may rapidly lead to the acquisition of drug resistance conclusively discouraging clinical intervention with targeted therapies such as GSK3β inhibitors (Marchand et al., 2015; Downward, 2015; Cox et al., 2014).

Novel epigenetic and transcriptomic studies allowed the classification of PDAC into two main subtypes according to their gene expression profiles, hence providing a prediction of chemoresistance as well as the prognosis (Chan-Seng-Yue et al., 2020). As mentioned above, concomitant inhibition of HDAC and GSK3β may prevent the emergence of drug resistance (Edderkaoui et al., 2018). More recently, new analyses were performed to define chromatin accessibility regions to identify epigenetics hallmarks of tumors sensitive to GSK3 inhibition; they reported that increased access to intronic and distal promoters regulating WNT cascade genes, as well as enrichment in transcription factor motifs, may result in WNT cascade amplification and drug resistance (Bruton et al., 2020). These findings, combined with early results from the prospective COMPASS study (NCT02750657), confirmed that chromatin profiling in advanced PDAC may help define tumors that could benefit from target therapies (Chan-Seng-Yue et al., 2020; Aung et al., 2018).

Specifically, Bruton and colleagues suggested that pancreatic tumors with high mutational burden and chromatin instability are more prone to develop drug resistance. Moreover, those tumors harboring loss of hepatocyte nuclear factor 4-α (HNF4α), an important regulator of endodermal lineage differentiation, more likely maintain sensitivity to GSK3β inhibitors, due to GSK3β upregulation and consequent increased tumor dependency (Bruton et al., 2020). Furthermore, ATAC-seq analysis on sensitive pancreatic tumors revealed mutations on chromatin modulators, possibly KFM6A, SETD2, MLL3, ARID1A and SETBP1, that favor distal promoter usage and alterations in either the AMPK signaling activator LKB1, or in WNT canonical pathway transducer LRP6. This proves their crucial role in the maintenance of cancer cell response to GSK3β-targeted monotherapy.

Many studies clearly showed the complex heterogeneity of pancreatic tumors and their microenvironment aiming to link the tumor genetic mapping with the prediction of patients' response to tailored treatment approaches (Bailey et al., 2016; Boyd et al., 2021). Determining the constellation of tumor genetic and transcriptional alterations not only helps the definition of specific cancer subtypes, but might also have implications to the development of targeted therapeutic strategies specifically designed to address the patient tumor profiles as well as to circumvent or surmount drug resistance.

3. Discussion and conclusions

Pancreatic cancer is a growing global health concern with an increasing incidence-to-mortality ratio. To date, it is the third most common cancer and, due to its frequent dismal prognosis, it will probably become the second-leading cause of cancer-related death in Western countries by 2030 (Rahib et al., 2014). The high genomic complexity and heterogeneity of pancreatic cancer, as well as its intrinsic metastatic behavior, represent an important barrier for the successful treatment of this lethal disease (Boyd et al., 2021). Surgical resection constitutes the only modest chance of cure, while actually standard-of-care therapeutic options are often palliative and offer an average of 5-years survival with most of patients developing drug resistance during the course of the treatment (Zeng et al., 2019). The poor outcomes are mainly related to late diagnosis and the strong aggressive nature of this malignant disease, highlighting the demand for discovering novel tumor vulnerabilities and effective therapies.

Despite dismal statistics, significant progress has been made to elucidate the molecular mechanisms involved in pancreatic cancer progression and chemoresistance. Among all, the glycogen synthase kinase-3 β, a highly conserved isoform of serine-threonine kinase GSK-3 family, has been recently found as an important determinant of PDAC onset and progression (Ougolkov et al., 2006; Zhang et al., 2011;). Initially described as a crucial modulator of glycogen synthesis, GSK3β is now confirmed to be involved in many fundamental cellular processes. Indeed, aberrant GSK3β activity has been implicated in different human disorders including bipolar depression, neurodegenerative disorders, acute myeloid leukemia, as well as many other malignancies (Walz et al., 2017; Hooper et al., 2008; Martelli et al., 2021).

Pre-clinical studies showed that pharmacologic inhibition or genetic depletion of GSK3β drastically reduced cell proliferation and cell survival of multiple human tumor types, further highlighting GSK3β as an attractive pharmacological target for therapeutic interventions against cancer (Rizzieri et al., 2016; Kotliarova et al., 2008; Korur et al., 2009; Song et al., 2010; Cohen et al., 1998; Carter et al., 2014; Kroon et al., 2014). Moreover, several studies on drug combination in patients with refractory solid tumors have shown that inhibition of GSK3β sensitizes resistant cancer cells to standard chemotherapeutic agents such as gemcitabine, nab-paclitaxel, doxorubicin, and FOLFIRINOX (Bhat et al., 2003; Kunnimalaiyaan et al., 2015; Shimasaki et al., 2012;). Thus, multiple evidence suggested that targeting GSK3β may reverse chemoresistance and highlighted its key role in many intracellular signaling pathways. These brought GSK3β to the attention of many researchers which are currently attempting to better understand the role of this this enigmatic kinase in the cellular dynamics of pancreas tumorigenesis and drug resistance.

Although GSK3β has been previously described as a tumor suppressor regulating the activity of numerous pro-oncogenic molecules such as c-Myc, β-catenin, cyclin D and c-Jun, a series of consistent observational studies reported that GSK3β is strongly upregulated in PDAC cells and could sustain pancreatic tumorigenesis (Kockeritz et al., 2006; Nagini et al., 2019; Walz et al., 2017). Notably, mutant KRas pancreatic cancers, accounting for 91 % of overall PDAC patients, present the overexpression and nuclear accumulation of active GSK3β which often correlate with poorly differentiated tumor state and poor outcomes (Christenson et al., 2016).

Tumors expressing mutated Ras usually harbor enhanced activation of mitogenic PI3k signaling and perturbed PTEN phosphatase activity, providing crucial signals driving tumor formation and maintenance (McCubrey et al., 2012a, 2012b; Fitzgerald et al., 2015; Waters and Channing, 2018). However, the intricate role of KRas in sustaining multiple mitogenic signaling pathways, such as activation of Akt, HER2 and EGFR, may explain the lack of success in developing KRas targeted therapies, despite decades of intense research efforts. More intriguing, GSK3β seems to be fundamental for the survival and growth of KRas-driven PDAC. Pharmacologic inhibition of GSK3β with SB, Tideglusib, AZD1080 and BIO, selectively reduced the proliferation of PDAC with dependency on mutant KRas, further evidencing the pro-survival effect of GSK3β in these tumors.

The controversial anti-tumorigenic or pro-tumorigenic role of GSK3β in PDAC is finely regulated by diverse mechanisms, including post-translational modifications, cellular localization and trafficking, formation of protein complexes, and substrate priming. All these processes have been extensively studied in order to understand the dynamics governing GSK3β activation and disruption.

Among all, phosphorylation of tyrosine 216, located within the conserved activation loop, is responsible for the full activation and kinase function of GSK3β, while serine 9 residue in the N-terminal lobe inhibits GSK3β activity when phosphorylated by other kinases (Cormier and Woodgett, 2017; Sutherland, 2011; Frame et al., 2001). However, considering pGSK3β-S9 as the inactive and pGSK3β-Y216 as the active form is probably over simplistic. In fact, GSK3β undergoes a dynamic equilibrium within those and others recently identified phosphorylation status in concert with stimulatory signaling molecules and primed substrate concentration. Additionally, serine 9 phosphorylation does not completely abrogate the catalytic activity of this kinase, as proven by the pGSK3β-S9-mediated phosphorylation of Gli3 within the Hh signaling pathway (Fitzgerald et al., 2015).

These observations might explain why many ATP-competitive GSK3β inhibitors interacting with the N-terminal lobe exert high concentration IC_{50} values and low kinase selectivity when compared to covalent inhibitors or non-ATP competitors. In fact, the ATP-binding domain is structurally conserved among most of kinases and, therefore, it is not surprising that some ATP competitive GSK3β inhibitors target also CDKs and other kinases which share a good degree of homology. Moreover, ATP-competitors increasing the inhibitory phosphorylation on serine-9 might not be optimal to abrogate GSK3β activity since phosphorylation of this residue, located within the binding pocket for primed-substrates recognition, might still result in GSK3β-mediated regulation of non-pre-phosphorylated targets.

On the other hand, covalent- or non-ATP-competitive inhibitors of GSK3β such as tideglusib, display moderate-to-weak binding but improved selectivity and low drug concentrations are required to attain therapeutic effects. Overall, these factors might determine the choice of using these classes of GSK3β inhibitors in clinical practice, but further studies on GSK-3 protein-substrate are required for future development of portent GSK3β inhibitors.

The promising therapeutic results expected from GSK3β targeting in PDAC found a solid base in the progressive increase of GSK3β expression which, in turn, strongly regulates NF-kB transcriptional activity (Ougolkov et al., 2006; Demarchi et al., 2003; Ben-Josef et al., 2015). This consequently triggered the stimulation of pathways involved in cell survival, proliferation and a pro-invasive transformation of pancreatic cancer cells, as well as in the promotion of Bim family proteins expression (Marchand et al., 2012).

Moreover, GSK3β appears to have a negative regulatory role on apoptosis, through phosphorylation and subsequent inactivation of pro-apoptotic molecules such as Bad, Ikk β and caspase 9, further sustaining a pro-survival phenotype (Cervello et al., 2012; Meng et al., 2018). GSK3β also modulates Wnt/β-catenin signaling: active GSK3β phosphorylates β-catenin, targeting it for ubiquitin-mediated proteasomal degradation (McCubrey et al., 2016) mediating Wnt cascade activation. This leads to inhibition of GSK3β activity, β-catenin accumulation and translocation into the nucleus, causing the expression of proto-oncogenes such as c-Myc and cyclin D1, along with genes promoting cell invasion and migration. In this regard, whether GSK3β action is dependent or not on β-catenin perturbation is still controversial, sustained WNT cascade activity was observed to drive drug resistance in PDAC models treated with GSK3β inhibitors (Freland and Beaulieu, 2012; Zhang et al., 2003; Yu et al., 2012).

To date, further mechanisms of drug resistance have been identified to be provoked by treatment with GSK3β inhibitors, mainly involving

the activation of autophagy/lysosomal network (Marchand et al., 2015). Although coexistence of both apoptosis and autophagic responses have been observed in many *in vitro* studies testing different GSK3β inhibitors, additional molecular studies reported that the transcription factor EB provided pro-survival autophagic signals by enhancing autophagy/lysosomal activity. Concomitantly, TFEB-depleted PDAC cells exhibited enhanced sensitivity to cell death upon GSK3β inhibition, further proving the protective role of autophagy in pancreatic cancer cells under GSK3β disruption. Similar results were observed in prostate cancer models, where GSK3β depletion enhanced AMP/ATP ratio, eliciting AMPK signals and autophagy activation (Sun et al., 2016). Even if combinatorial inhibition of GSK3β and TFED has not been investigated yet, assessing GSK3β-mediated regulation of autophagic responses might be a future achievement in the development of PDAC treatment.

To prevent the onset of drug resistance, novel advances in understanding escape pathways and chromatin landscape in GSK3β inhibition have been elucidated in experimental settings. Epigenetic analysis revealed that histone deacetylases are highly expressed in PDAC cells, coordinating cell cycle progression, differentiation and apoptosis. Enhanced HDACs activity, resulting from GSK3β-mediated activation of Zeb1 transcription factors, consequently induced the repression of E-cadherin expression and lead to a poorer prognosis (Aghdassi et al., 2012). Indeed, concomitant inhibition of GSK3β and HDACs with the synthetic agent Metavert caused synergistic anti-proliferative effects on PDAC cells and prevented EMT-associated gene expression (Edderkaoui et al., 2018).

Furthermore, chromatin profiling among advanced pancreatic cancer suggests that tumors harboring high mutational burden and chromatin instability are more prone to present drug resistance, and patients with loss of the hepatocyte nuclear factor 4-α and enhanced GSK3β expression were more likely to respond to treatment with GSK3β inhibitors (Bruton et al., 2020).

A general optimism is increasing nowadays when combining chemotherapy with novel agents, targeting specific features of different tumors. Indeed, it is overall admitted that drugs targeting various tumor-survival signaling cascades might exert therapeutic advantages compared to single agent treatment approaches. Therefore, considerable advances have been achieved in the development of targeted drugs with reduced toxicities, improved survival benefits and potentiality to overcome or prevent chemoresistance.

However, approved targeted therapies for pancreatic cancer treatment include only olaparib (Lynparza), and erlotinib (Tarceva). Recent preclinical studies underlined the potential of new inhibitors of the focal adhesion kinase (Le Large et al., 2021) or c-Met (Firuzi et al., 2019). Similarly, a few studies showed that administration of GSK3β inhibitors reduced pancreatic cancer cell proliferation, but more interestingly, it resensitized drug resistant cells to standard of care chemotherapy within combinatorial regimens. Indeed, disruption of GSK3β has been described to modulate the TAK1-YAP/TAZ axis and the ATR-mediated DNA damage response pathway, thus driving the restoration of effective cytotoxic response (Ding et al., 2019; Lin et al., 2015).

On the other hand, GSK3β is a fundamental crossroad for multiple anti-oncogenic pathways, by promoting tumor suppressor signaling cascades and this feature has raised a general mistrust in approving such agents for therapeutic interventions against cancer. Thus, the evaluation of GSK3β inhibitors in clinical trials has been hampered by the concern that inhibition of GSK3β may stimulate malignant transformation. However, future promising perspectives for GSK3β inhibitors clinical management of cancer has been recently achieved from observational studies reporting that long-term use of the only approved GSK3 inhibitor, lithium, is not associated with increased risk of cancer in patients with bipolar disorder (Martinsson et al., 2016)

Overall, developing the medical treatment of choice for tumors, such as PDAC, that manifests high recurrence and persistent invasion capacity, metastasis and development of drug tolerance, remains a challenge for current clinical interventions. Chemotherapeutic interventions, radiation and immunotherapy have indeed minimal effect on patient's survival, highlighting the burning need for additional mechanistic studies exploiting cellular vulnerabilities of advanced and metastatic PDAC. In this respect, additional studies are required to extensively understand the consequence and dynamics regulating aberrant GSK3β activity. Furthermore, a specific focus should also be directed to GSK-3α, the other isoform of GSK3, which presents distinctive cellular functions. Since these kinases are differentially expressed within tissues and the majority of cancer studies has focused on GSK3β, it is questionable if targeting GSK3α together with GSK3β has a major effect than single GSK-3β inhibition.

In conclusion, the combination of GSK3β inhibitors with chemotherapy is strategically poised to be a promising approach to overcome the emergence of early drug resistance or to overcome chemoresistance in advanced and metastatic pancreatic tumors. Further understanding of the dynamics governing PDAC tumorigenesis and cancer progression involving GSK3β, might help the achievement of clinical strategies aimed at ameliorating survival benefits and to convert this deadly tumor into a more manageable chronic malignancy.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.drup.2021.100779.

References

Abrams, S.L., Akula, S.M., Meher, A.K., Steelman, L.S., Gizak, A., Duda, P., Rakus, D., Martelli, A.M., Ratti, S., Cocco, L., Montalto, G., Cervello, M., Ruvolo, P., Libra, M., Falzone, L., Candido, S., McCubrey, J.A., 2021. GSK-3β can regulate the sensitivity of MIA-PaCa-2 pancreatic and MCF-7 breast cancer cells to chemotherapeutic drugs, targeted therapeutics and nutraceuticals. Cells 10 (4), 816. https://doi.org/10.3390/cells10040816.

Aghdassi, A., Sendler, M., Guenther, A., Mayerle, J., Behn, C.O., Heidecke, C.D., Friess, H., Büchler, M., Evert, M., Lerch, M.M., Weiss, F.U., 2012. Recruitment of histone deacetylases HDAC1 and HDAC2 by the transcriptional repressor ZEB1 downregulates E-cadherin expression in pancreatic cancer. Gut 61, 439–448.

Arlt, A., Gehrz, A., Müerköster, S., Vorndamm, J., Kruse, M.L., Fölsch, U.R., Schäfer, H., 2003. Role of NF-kappaB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine-induced cell death. Oncogene 22, 3243–3251.

Arora, S., Bhardwaj, A., Singh, S., Srivastava, S.K., McClellan, S., Nirodi, C.S., Piazza, G. A., Grizzle, W.E., Owen, L.B., Singh, A.P., 2013. An undesired effect of chemotherapy: gemcitabine promotes pancreatic cancer cell invasiveness through reactive oxygen species-dependent, nuclear factor κB- and hypoxia-inducible factor 1α-mediated up-regulation of CXCR4. J. Biol. Chem. 288, 21197–21207.

Aung, K.L., Fischer, S.E., Denroche, R.E., Jang, G.H., Dodd, A., Creighton, S., Southwood, B., Liang, S.B., Chadwick, D., Zhang, A., O'Kane, G.M., Albaba, H., Moura, S., Grant, R.C., Miller, J.K., Mbabaali, F., Pasternack, D., Lungu, I.M., Bartlett, J.M.S., Ghai, S., Lemire, M., Holter, S., Connor, A.A., Moffitt, R.A., Yeh, J.J., Timms, L., Krzyzanowski, P.M., Dhani, N., Hedley, D., Notta, F., Wilson, J.M., Moore, M.J., Gallinger, S., Knox, J.J., 2018. Genomics-driven precision medicine for advanced pancreatic cancer: early results from the COMPASS trial. Clin. Cancer Res. 24, 1344–1354.

Bailey, P., Chang, D., Nones, K., et al., 2016. Genomic analyses identify molecular subtypes of pancreatic cancer. Nature 531, 47–52.

Bang, D., Wilson, W., Ryan, M., Yeh, J.J., Baldwin, A.S., 2013. GSK-3α promotes oncogenic KRAS function in pancreatic cancer via TAK1-TAB stabilization and regulation of noncanonical NF-κB. Cancer Discov. 3, 690–703.

Baudino, T.A., 2015. Targeted cancer therapy: the next generation of cancer treatment. Curr. Drug Discov. Technol. 12, 3–20.

Baumgart, S., Chen, N.M., Zhang, J.S., Billadeau, D.D., Gaisina, I.N., Kozikowski, A.P., Singh, S.K., Fink, D., Ströbel, P., Klindt, C., Zhang, L., Bamlet, W.R., Koenig, A., Hessmann, E., Gress, T.M., Ellenrieder, V., Neesse, A., 2016. GSK-3β governs inflammation-induced NFATc2 signaling hubs to promote pancreatic cancer progression. Mol. Cancer Ther. 15, 491–502.

Ben-Josef, George, A., Regine, W.F., Abrams, R., Morgan, M., Thomas, D., Schaefer, P.L., DiPetrillo, T.A., Fromm, M., Small Jr., W., Narayan, S., Winter, K., Griffith, K.A., Guha, C., T.M, 2015. Williams Glycogen synthase kinase 3 beta predicts survival in resected adenocarcinoma of the pancreas. Clin. Cancer Res. 21, 5612–5618.
Beurel, E., Grieco, S.F., Jope, R.S., 2015. Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. Pharmacol. Ther. 0, 114–131.
Bhat, R., Xue, Y., Berg, S., Hellberg, S., Ormö, M., Nilsson, Y., Radesäter, A.C., Jerning, E., Markgren, P.O., Borgegård, T., Nylöf, M., Giménez-Cassina, A., Hernández, F., Lucas, J.J., Díaz-Nido, J., Avila, J., 2003. Structural insights and biological effects of glycogen synthase kinase 3-specific inhibitor AR-A014418. J. Biol. Chem. 278, 45937–45945.
Bijur, G.N., Jope, R.S., 2003. Glycogen synthase kinase-3 beta is highly activated in nuclei and mitochondria. Neuroreport 14, 2415–2419.
Binenbaum, Y., Na'ara, S., Gil, Z., 2015. Gemcitabine resistance in pancreatic ductal adenocarcinoma. Drug Resist. Updat. 23, 55–68.
Bowden, C.L., 2000. Efficacy of lithium in mania and maintenance therapy of bipolar disorder. J. Clin. Psychiatry 61 (Suppl. 9), 35–40.
Boyd, L.N.C., Andini, K.D., Peters, G.J., Kazemier, G., Giovannetti, E., 2021. Heterogeneity and plasticity of cancer-associated fibroblasts in the pancreatic tumor microenvironment. Semin. Cancer Biol. 15. S1044-579X(21)00056-0.
Brunton, H., Caligiuri, G., Cunningham, R., et al., 2020. HNF4A and GATA6 loss reveals therapeutically actionable subtypes in pancreatic cancer. Cell Rep. 31 (6), 107625. https://doi.org/10.1016/j.celrep.2020.107625.
Caparello, C., Meijer, L.L., Garajova, I., Falcone, A., Le Large, T.Y., Funel, N., Kazemier, G., Peters, G.J., Vasile, E., Giovannetti, E., 2016. FOLFIRINOX and translational studies: towards personalized therapy in pancreatic cancer. World J. Gastroenterol. 22, 6987–7005.
Carbone, D., Parrino, B., Cascioferro, S., Pecoraro, C., Giovannetti, E., Di Sarno, V., Musella, S., Auriemma, G., Cirrincione, G., Diana, P., 2021. 1,2,4-oxadiazole topsentin analogs with antiproliferative activity against pancreatic cancer cells, targeting GSK3β kinase. ChemMedChem 16, 537–554.
Carneiro, B.A., Anraku, T., Kazama, A., Bilim, V., Tasaki, M., Schmitt, D., Mazar, A.P., Giles, F.J., Ugolkov, A., Tomita, Y., 2020. Phase I study of 9-ing-41, a small molecule selective glycogen synthase kinase-3 beta (GSK-3β) inhibitor, as a single agent and combined with chemotherapy, in patients with refractory tumors. J. Clin. Oncol. 38, 3507.
Carter, Y.M., Kunnimalaiyaan, S., Chen, H., Gamblin, T.C., Kunnimalaiyaan, M., 2014. Specific glycogen synthase kinase-3 inhibition reduces neuroendocrine markers and suppresses neuroblastoma cell growth. Cancer Biol. Ther. 15, 510–515.
Cervello, M., McCubrey, J.A., Cusimano, A., Lampiasi, N., Azzolina, A., Montalto, G., 2012. Targeted therapy for epatocellular carcinoma: novel agents on the horizon. Oncotarget 3, 236–260.
Chan-Seng-Yue, M., Kim, J.C., Wilson, G.W., et al., 2020. Transcription phenotypes of pancreatic cancer are driven by genomic events during tumor evolution. Nat. Genet. 52, 231–240.
Chen, S., Sun, K.X., Feng, M.X., Sang, X.B., Liu, B.L., Zhao, Y., 2016. Role of glycogen synthase kinase-3β inhibitor AZD1080 in ovarian cancer. Drug Des. Devel. Ther. 10, 1225–1232.
Christenson, E.S., Jaffee, E., Azad, N.S., 2016. Current and emerging therapies for patients with advanced pancreatic ductal adenocarcinoma: a bright future. Lancet Oncol. 21 (3), e135–e145.
Cohen, Y., Chetrit, A., Cohen, Y., Sirota, P., Modan, B., 1998. Cancer morbidity in psychiatric patients: influence of lithium carbonate treatment. Med. Oncol. Northwood Lond. Engl. 15, 32–36.
Collisson, E.A., Bailey, P., Chang, D.K., Biankin, A.V., 2019. Molecular subtypes of pancreatic cancer. Nat. Rev. Gastroenterol. Hepatol. 16, 207–220.
Coppola, S., Carnevale, I., Danen, E.H.J., Peters, G.J., Schmidt, T., Assaraf, Y.G., Giovannetti, E., 2017. A mechanopharmacology approach to overcome chemoresistance in pancreatic cancer. Drug Resist. Update 31, 43–51.
Cormier, K.W., Woodgett, J.R., 2017. Recent advances in understanding the cellular roles of GSK-3. F1000Research 6.
Cox, A.D., Fesik, S.W., Kimmelman, A.C., Luo, J., Der, C.J., 2014. Drugging the undruggable Ras: mission possible? Nat. Rev. Drug Discov. 13, 828–851.
Dajani, R., Fraser, E., Roe, S.M., Yeo, M., Good, V.M., Thompson, V., Dale, T.C., Pearl, L. H., 2003. Structural basis for recruitment of glycogen synthase kinase 3beta to the axin-APC scaffold complex. EMBO J. 22, 494–501.
De Sarno, P., Li, X., Jope, R.S., 2002. Regulation of Akt and glycogen synthase kinase-3 beta phosphorylation by sodium valproate and lithium. Neuropharmacology 43, 1158–1164.
Demarchi, F., Bertoli, C., Sandy, P., Schneider, C., 2003. Glycogen synthase kinase-3 beta regulates NF-kappa B1/p105 stability. J. Biol. Chem. 278, 39583–39590.
Dewi, F.R.P., Domoto, T., Hazawa, M., Kobayashi, A., Douwaki, T., Minamoto, T., Wong, R.W., 2018. Colorectal cancer cells require glycogen synthase kinase-3β for sustaining mitosis via translocated promoter region (TPR)-dynein interaction. Oncotarget 9, 13337–13352.
Ding, L., Billadeau, D.D., 2020. Glycogen synthase kinase-3β: a novel therapeutic target for pancreatic cancer. Expert Opin. Ther. Targets 24, 417–426.
Ding, L., Liou, G.Y., Schmitt, D.M., Storz, P., Zhang, J.S., Billadeau, D.D., 2017. Glycogen synthase kinase-3β ablation limits pancreatitis-induced acinar-to-ductal metaplasia. J. Pathol. 243, 65–77. https://doi.org/10.1002/path.4928.
Ding, L., Madamsetty, V.S., Kiers, S., Alekhina, O., Ugolkov, A., Dube, J., Zhang, Y., Zhang, J.S., Wang, E., Dutta, S.K., Schmitt, D.M., Giles, F.J., Kozikowski, A.P., Mazar, A.P., Mukhopadhyay, D., Billadeau, D.D., 2019. Glycogen synthase kinase-3 inhibition sensitizes pancreatic cancer cells to chemotherapy by abrogating the TopBP1/ATR-mediated DNA damage response. Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 25, 6452–6462.
Doble, B.W., Woodgett, J.R., 2003. GSK-3: tricks of the trade for a multi-tasking kinase. J. Cell. Sci. 116, 1175–1186.
Doble, B.W., Woodgett, J.R., 2007. Role of glycogen synthase kinase-3 in cell fate and epithelial-mesenchymal transitions. Cells Tissues Organs (Print) 185, 73–84.
Domínguez, J.M., Fuertes, A., Orozco, L., del Monte-Millán, M., Delgado, E., Medina, M., 2012. Evidence for irreversible inhibition of glycogen synthase kinase-3β by tideglusib. J. Biol. Chem. 287, 893–904. https://doi.org/10.1074/jbc.M111.306472.
Domoto, T., Pyko, I.V., Furuta, T., Miyashita, K., Uehara, M., Shimasaki, T., Nakada, M., Minamoto, T., 2016. Glycogen synthase kinase-3β is a pivotal mediator of cancer invasion and resistance to therapy. Cancer Sci. 107, 1363–1372.
Domoto, T., Uehara, M., Bolidong, D., Minamoto, T., 2020. Glycogen synthase kinase 3β in cancer biology and treatment. Cells 9.
Downward, J., 2015. RAS synthetic lethal screens revisited: still seeking the elusive prize? Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 21, 1802–1809.
Duda, P., Akula, S.M., Abrams, S.L., Steelman, L.S., Martelli, A.M., Cocco, L., Ratti, S., Candido, S., Libra, M., Montalto, G., Cervello, M., Gizak, A., Rakus, D., McCubrey, J. A., 2020. Targeting GSK3 and associated signaling pathways involved in cancer. Cells 9.
Edderkaoui, M., Chheda, C., Soufi, B., Zayou, F., Hu, R.W., Ramanujan, V.K., Pan, X., Boros, L.G., Tajbakhsh, J., Madhav, A., Bhowmick, N.A., Wang, Q., Lewis, M., Tuli, R., Habtezion, A., Murali, R., Pandol, S.J., 2018. An inhibitor of GSK3B and HDACs kills pancreatic cancer cells and slows pancreatic tumor growth and metastasis in mice. Gastroenterology 155. https://doi.org/10.1053/j.gastro.2018.08.028, 1985-1998.e5.
El Hassouni, B., Li Petri, G., Liu, D., Cascioferro, S., Parrino, B., Hassan, W., Diana, P., Ali, A., Frampton, A.E., Giovannetti, E., 2019. Pharmacogenetics of treatments for pancreatic cancer. Expert Opin. Drug Metab. Toxicol. 15, 437–447.
Eldar-Finkelman, H., Martinez, A., 2011. GSK-3 inhibitors: preclinical and clinical focus on CNS. Front. Mol. Neurosci. 4.
Elmaci, İ., Altinoz, M.A., 2016. A metabolic inhibitory cocktail for grave cancers: metformin, pioglitazone and Lithium combination in treatment of pancreatic cancer and glioblastoma multiforme. Biochem. Genet. 54, 573–618.
Eser, S., Schnieke, A., Schneider, G., Saur, D., 2014. Oncogenic KRAS signalling in pancreatic cancer. Br. J. Cancer 111, 817–822.
Fang, X., Yu, S.X., Lu, Y., Bast Jr, R.C., Woodgett, J.R., Mills, G.B., 2000. Phosphorylation and inactivation of glycogen synthase kinase 3 by protein kinase A. Proc. Natl. Acad. Sci. U. S. A. 97, 11960–11965.
Firuzi, O., Che, P.P., El Hassouni, B., Buijs, M., Coppola, S., Löhr, M., Funel, N., Heuchel, R., Carnevale, I., Schmidt, T., Mantini, G., Avan, A., Saso, L., Peters, G.J., Giovannetti, E., 2019. Role of c-MET inhibitors in overcoming drug resistance in spheroid models of primary human pancreatic cancer and stellate cells. Cancers (Basel) 11 (5), 638.
Fitzgerald, T.L., Lertpiriyapong, K., Cocco, L., Martelli, A.M., Libra, M., Candido, S., Montalto, G., Cervello, M., Steelman, L., Abrams, S.L., et al., 2015. Roles of EGFR and KRAS and their downstream signaling pathways in pancreatic cancer and pancreatic cancer stem cells. Adv. Biol. Regul. 59, 65–81.
Fleming, J.B., Shen, G.-L., Holloway, S.E., Davis, M., Brekken, R.A., 2005. Molecular consequences of silencing mutant K-ras in pancreatic cancer cells: justification for K-ras-directed therapy. Mol. Cancer Res. MCR 3, 413–423. https://doi.org/10.1158/1541-7786.MCR-04-0206.
Foltz, D.R., Santiago, M.C., Berechid, B.E., Nye, J.S., 2002. Glycogen synthase kinase-3beta modulates notch signaling and stability. Curr. Biol. CB 12, 1006–1011.
Frame, S., Cohen, P., Biondi, R.M., 2001. A common phosphate binding site explains the unique substrate specificity of GSK3 and its inactivation by phosphorylation. Mol. Cell 7, 1321–1327.
Freland, L., Beaulieu, J.-M., 2012. Inhibition of GSK3 by lithium, from single molecules to signaling networks. Front. Mol. Neurosci. 5.
Gaisina, I.N., Gallier, F., Ougolkov, A.V., Kim, K.H., Kurome, T., Guo, S., Holzle, D., Luchini, D.N., Blond, S.Y., Billadeau, D.D., Kozikowski, A.P., 2009. From a natural product lead to the identification of potent and selective benzofuran-3-yl-(indol-3-yl) maleimides as glycogen synthase kinase 3beta inhibitors that suppress proliferation and survival of pancreatic cancer cells. J. Med. Chem. 52, 1853–1863.
Galluzzi, L., Pietrocola, F., Bravo-San Pedro, J.M., Amaravadi, R.K., Baehrecke, E.H., Cecconi, F., Codogno, P., Debnath, J., Gewirtz, D.A., Karantza, V., Kimmelman, A., Kumar, S., Levine, B., Maiuri, M.C., Martin, S.J., Penninger, J., Piacentini, M., Rubinsztein, D.C., Simon, H.U., Simonsen, A., Thorburn, A.M., Velasco, G., Ryan, K. M., Kroemer, G., 2015. Autophagy in malignant transformation and cancer progression. EMBO J. 34, 856–880.
Gao, X., Wang, J.-Y., Gao, L.-M., Yin, X.-F., Liu, L., 2013. Identification and analysis of glycogen synthase kinase 3 beta1 interactome. Cell Biol. Int. 37, 768–779.
Garcea, G., Manson, M.M., Neal, C.P., Pattenden, C.J., Sutton, C.D., Dennison, A.R., Berry, D.P., 2007. Glycogen synthase kinase-3 beta; a new target in pancreatic cancer. Curr. Cancer Drug Targets 7, 209–215. https://doi.org/10.2174/156800907780618266.
Garcia-Sampedro, A., Gaggia, G., Ney, A., Mahamed, I., Acedo, P., 2021. The state-of-the-Art of phase II/III clinical trials for targeted pancreatic cancer therapies. J. Clin. Med. 10, 566.
Giovannetti, E., Wang, Q., Avan, A., Funel, N., Lagerweij, T., Lee, J.H., Caretti, V., van der Velde, A., Boggi, U., Wang, Y., Vasile, E., Peters, G.J., Wurdinger, T., Giaccone, G., 2014. CYB5A role in pancreatic cancer prognosis and autophagy modulation. JNCI – J. Natl. Cancer Inst. 106, djt346.
Giovannetti, E., van der Borden, C.L., Frampton, A.E., Ali, A., Firuzi, O., Peters, G.J., 2017. Never let it go: stopping key mechanisms underlying metastasis to fight pancreatic cancer. Semin. Cancer Biol. 44, 43–59.

Gomez Mellado, V., Giovannetti, E., Peters, G.J., 2015. Unraveling the complexity of autophagy: potential therapeutic applications in Pancreatic Ductal Adenocarcinoma. Sem Cancer Biol. 35, 11–19.
Gray, J.E., Infante, J.R., Brail, L.H., Simon, G.R., Cooksey, J.F., Jones, S.F., Farrington, D. L., Yeo, A., Jackson, K.A., Chow, K.H., Zamek-Gliszczynski, M.J., Burris, H.A., 2015. A first-in-human phase I dose-escalation, pharmacokinetic, and pharmacodynamic evaluation of intravenous LY2090314, a glycogen synthase kinase 3 inhibitor, administered in combination with pemetrexed and carboplatin. Invest. New Drugs 33, 1187–1196.
Guo, D., Shen, Y., Li, W., Li, Q., Zhao, Y., Pan, C., Chen, B., Zhong, Y., Miao, Y., 2019. 6-bromoindirubin-3′-Oxime (6BIO) suppresses the mTOR pathway, promotes autophagy, and exerts anti-aging effects in rodent liver. Front. Pharmacol. 10.
Hermida, M.A., Dinesh Kumar, J., Leslie, N.R., 2017. GSK3 and its interactions with the PI3K/AKT/mTOR signalling network. Adv. Biol. Regul. 65, 5–15.
Hill, A., Chung, V., 2020. Pancreatic cancer. In: Salgia, R. (Ed.), Oncology in the Precision Medicine Era. Springer, Cham.
Hoeflich, K.P., Luo, J., Rubie, E.A., Tsao, M.S., Jin, O., Woodgett, J.R., 2000. Requirement for glycogen synthase kinase-3beta in cell survival and NF-kappaB activation. Nature 406, 86–90. https://doi.org/10.1038/35017574.
Holcomb, B., Yip-Schneider, M., Schmidt, C.M., 2008. The role of nuclear factor kappaB in pancreatic cancer and the clinical applications of targeted therapy. Pancreas 36, 225–235.
Hooper, C., Killick, R., Lovestone, S., 2008. The GSK3 hypothesis of Alzheimer's disease. J. Neurochem. 104, 1433–1439.
Horrigan, J., Gomes, T.B., Snape, M., Nikolenko, N., McMorn, A., Evans, S., Yaroshinsky, A., Della Pasqua, O., Oosterholt, S., Lochmüller, H., 2020. A phase 2 study of AMO-02 (Tideglusib) in congenital and childhood-onset myotonic dystrophy type 1 (DM1). Pediatr. Neurol. 112, 84–93.
Hughes, K., Nikolakaki, E., Plyte, S.E., Totty, N.F., Woodgett, J.R., 1993. Modulation of the glycogen synthase kinase-3 family by tyrosine phosphorylation. EMBO J. 12, 803–808.
Ireland, L., Santos, A., Ahmed, M.S., Rainer, C., Nielsen, S.R., Quaranta, V., Weyer-Czernilofsky, U., Engle, D.D., Perez-Mancera, P.A., Coupland, S.E., Taktak, A., Bogenrieder, T., Tuveson, D.A., Campbell, F., Schmid, M.C., Mielgo, A., 2016. Chemoresistance in pancreatic cancer is driven by stroma-derived insulin-like growth factors. Cancer Res. 76, 6851–6863.
Johnson, F.N., Amdisen, A., 1983. The first era of lithium in medicine. An historical note. Pharmacopsychiatria 16, 61–63.
Kaidanovich-Beilin, O., Woodgett, J.R., 2011. GSK-3: functional insights from cell biology and animal models. Front. Mol. Neurosci. 4.
Kaltschmidt, B., Greiner, J.F.W., Kadhim, H.M., Kaltschmidt, C., 2018. Subunit-specific role of NF-κB in cancer. Biomedicines 6, 44.
Kaur, S., Smith, L.M., Patel, A., Menning, M., Watley, D.C., Malik, S.S., Krishn, S.R., Mallya, K., Aithal, A., Sasson, A.R., Johansson, S.L., Jain, M., Singh, S., Guha, S., Are, C., Raimondo, M., Hollingsworth, M.A., Brand, R.E., Batra, S.K., 2017. A combination of MUC5AC and CA19-9 improves the diagnosis of pancreatic cancer: a multicenter study. Am. J. Gastroenterol. 112, 172–183.
Kazi, A., Xiang, S., Yang, H., Delitto, D., Trevino, J., Jiang, R.H.Y., Ayaz, M., Lawrence, H.R., Kennedy, P., Sebti, S.M., 2018. GSK3 suppression upregulates β-catenin and c-Myc to abrogate KRas-dependent tumors. Nat. Commun. 9, 5154.
Kim, K., Pang, K.M., Evans, M., Hay, E.D., 2000. Overexpression of beta-catenin induces apoptosis independent of its transactivation function with LEF-1 or the involvement of major G1 cell cycle regulators. Mol. Biol. Cell 11, 3509–3523.
Kitano, A., Shimasaki, T., Chikano, Y., Nakada, M., Hirose, M., Higashi, T., Ishigaki, Y., Endo, Y., Takino, T., Sato, H., Sai, Y., Miyamoto, K., Motoo, Y., Kawakami, K., Minamoto, T., 2013. Aberrant glycogen synthase kinase 3β is involved in pancreatic Cancer cell invasion and resistance to therapy. PLoS One 8.
Kockeritz, L., Doble, B., Patel, S., Woodgett, J.R., 2006. Glycogen synthase kinase-3–an overview of an over-achieving protein kinase. Curr. Drug Targets 7, 1377–1388.
Komiya, Y., Habas, R., 2008. Wnt signal transduction pathways. Organogenesis 4, 68–75. https://doi.org/10.4161/org.4.2.5851.
Korur, S., Huber, R.M., Sivasankaran, B., Petrich, M., Morin Jr., P., Hemmings, B.A., Merlo, A., Lino, M.M., 2009. GSK3beta regulates differentiation and growth arrest in glioblastoma. PLoS One 4, e7443.
Kotliarova, S., Pastorino, S., Kovell, L.C., Kotliarov, Y., Song, H., Zhang, W., Bailey, R., Maric, D., Zenklusen, J.C., Lee, J., Fine, H.A., 2008. Glycogen synthase kinase 3 inhibition induces glioma cell death through c-MYC, NF-κB and glucose regulation. Cancer Res. 68, 6643–6651.
Krause, U., Bertrand, L., Maisin, L., Rosa, M., Hue, L., 2002. Signalling pathways and combinatory effects of insulin and amino acids in isolated rat hepatocytes. Eur. J. Biochem. 269, 3742–3750.
Kroon, J., in' t Veld, L.S., Buijs, J.T., Cheung, H., van der Horst, G., van der Pluijm, G., 2014. Glycogen synthase kinase-3β inhibition depletes the population of prostate cancer stem/progenitor-like cells and attenuates metastatic growth. Oncotarget 5, 8986–8994.
Kunnimalaiyaan, S., Gamblin, T.C., Kunnimalaiyaan, M., 2015. Glycogen synthase kinase-3 inhibitor AR-A014418 suppresses pancreatic cancer cell growth via inhibition of GSK-3-mediated Notch1 expression. HPB 17, 770–776.
Kuroki, H., Anraku, T., Kazama, A., Bilim, V., Tasaki, M., Schmitt, D., Mazar, A.P., Giles, F.J., Ugolkov, A., Tomita, Y., 2019. 9-ING-41, a small molecule inhibitor of GSK-3beta, potentiates the effects of anticancer therapeutics in bladder cancer. Sci. Rep. 9, 19977.
Le Large, T.Y., Bijlsma, M.F., Kazemier, G., van Laarhoven, H.M., Giovannetti, E., Jimenez, C.R., 2017. Key biological processes driving metastatic spread of pancreatic cancer as identified by multi-omics studies. Semin. Cancer Biol. 44, 153–169.
Le Large, T.Y., Mantini, G., Meijer, L.L., Pham, T.V., Funel, N., van Grieken, N.C., Kok, B., Knol, J., van Laarhoven, H.W., Piersma, S.R., Jimenez, C.R., Kazemier, G., Giovannetti, E., Bijlsma, M.F., 2020. Microdissected pancreatic cancer proteomes reveal tumor heterogeneity and therapeutic targets. JCI Insight 5, 138290.
Le Large, T.Y.S., Bijlsma, M.F., El Hassouni, B., Mantini, G., Lagerweij, T., Henneman, A. A., Funel, N., Kok, B., Pham, T.V., de Haas, R., Morelli, L., Knol, J.C., Piersma, S.R., Kazemier, G., van Laarhoven, H.W.M., Giovannetti, E., Jimenez, C.R., 2021. Focal adhesion kinase inhibition synergizes with nab-paclitaxel to target pancreatic ductal adenocarcinoma. J. Exp. Clin. Cancer Res. 40, 91.
Li, M., Wang, X., Meintzer, M.K., Laessig, T., Birnbaum, M.J., Heidenreich, K.A., 2000. Cyclic AMP promotes neuronal survival by phosphorylation of glycogen synthase kinase 3beta. Mol. Cell. Biol. 20, 9356–9363.
Li, J., Gong, X., Jiang, R., Lin, D., Zhou, T., Zhang, A., Li, H., Zhang, X., Wan, J., Kuang, G., Li, H., 2018. Fisetin inhibited growth and metastasis of triple-negative breast cancer by reversing epithelial-to-mesenchymal transition via PTEN/Akt/GSK3β signal pathway. Front. Pharmacol. 9.
Liang, J., Slingerland, J.M., 2003. Multiple roles of the PI3K/PKB (Akt) pathway in cell cycle progression. Cell Cycle Georget. Tex 2, 339–345.
Lin, L., Sabnis, A.J., Chan, E., Olivas, V., Cade, L., Pazarentzos, E., Asthana, S., Neel, D., Yan, J.J., Lu, X., Pham, L., Wang, M.M., Karachaliou, N., Cao, M.G., Manzano, J.L., Ramirez, J.L., Torres, J.M., Buttitta, F., Rudin, C.M., Collisson, E.A., Algazi, A., Robinson, E., Osman, I., Muñoz-Couselo, E., Cortes, J., Frederick, D.T., Cooper, Z.A., McMahon, M., Marchetti, A., Rosell, R., Flaherty, K.T., Wargo, J.A., Bivona, T.G., 2015. The Hippo effector YAP promotes resistance to RAF- and MEK-targeted cancer therapies. Nat. Genet. 47, 250–256.
Liptay, S., Weber, C.K., Ludwig, L., Wagner, M., Adler, G., Schmid, R.M., 2003. Mitogenic and antiapoptotic role of constitutive NF-kappaB/Rel activity in pancreatic cancer. Int. J. Cancer 105, 735–746.
Liu, K., Li, J., Wu, X., Chen, M., Luo, F., Li, J., 2017. GSK-3β inhibitor 6-bromo-indirubin-3'-oxime promotes both adhesive activity and drug resistance in colorectal cancer cells. Int. J. Oncol. 51, 1821–1830.
Lomberk, G., Blum, Y., Nicolle, R., et al., 2018. Distinct epigenetic landscapes underlie the pathobiology of pancreatic cancer subtypes. Nat. Commun. 9, 1978.
Lovestone, S., Boada, M., Dubois, B., Hüll, M., Rinne, J.O., Huppertz, H.J., Calero, M., Andrés, M.V.-, Gómez-Carrillo, B., León, T., del Ser, T., 2015. A phase II trial of tideglusib in Alzheimer's disease. J. Alzheimers Dis. JAD 45, 75–88.
Mamaghani, S., Patel, S., Hedley, D.W., 2009. Glycogen synthase kinase-3 inhibition disrupts nuclear factor-kappaB activity in pancreatic cancer, but fails to sensitize to gemcitabine chemotherapy. BMC Cancer 9, 132.
Mamaghani, S., Simpson, C.D., Cao, P.M., Cheung, M., Chow, S., Bandarchi, B., Schimmer, A.D., Hedley, D.W., 2012. Glycogen synthase kinase-3 inhibition sensitizes pancreatic cancer cells to TRAIL-induced apoptosis. PLoS One 7, e41102.
Marchand, B., Tremblay, I., Cagnol, S., Boucher, M.-J., 2012. Inhibition of glycogen synthase kinase-3 activity triggers an apoptotic response in pancreatic cancer cells through JNK-dependent mechanisms. Carcinogenesis 33, 529–537.
Marchand, B., Arsenault, D., Raymond-Fleury, A., Boisvert, F.-M., Boucher, M.-J., 2015. Glycogen synthase Kinase-3 (GSK3) inhibition induces prosurvival autophagic signals in human pancreatic cancer cells*. J. Biol. Chem. 290, 5592–5605.
Martelli, A.M., Evangelisti, C., Paganelli, F., Chiarini, F., McCubrey, J.A., 2021. GSK-3: a multifaceted player in acute leukemias. Leukemia 1–14.
Martinsson, L., Westman, J., Hällgren, J., Ösby, U., Backlund, L., 2016. Lithium treatment and cancer incidence in bipolar disorder. Bipolar Disord. 18 (1), 33–40.
Massihnia, D., Avan, A., Funel, N., Maftouh, M., van Krieken, A., Granchi, C., Raktoe, R., Boggi, U., Aicher, B., Minutolo, F., Russo, A., Leon, L.G., Peters, G.J., Giovannetti, E., 2017. Phospho-Akt overexpression is prognostic and can be used to tailor the synergistic interaction of Akt inhibitors with gemcitabine in pancreatic cancer. J. Hematol. Oncol. 10, 9.
McCubrey, J.A., Steelman, L.S., Chappell, W.H., Abrams, S.L., Franklin, R.A., Montalto, G., Cervello, M., Libra, M., Candido, S., Malaponte, G., et al., 2012a. Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR cascade inhibitors: how mutations can result in therapy resistance and how to overcome resistance. Oncotarget 3, 1068–1111.
McCubrey, J.A., Steelman, L.S., Chappell, W.H., Abrams, S.L., Franklin, R.A., Montalto, G., Cervello, M., Libra, M., Candido, S., Malaponte, G., et al., 2012b. Mutations and deregulation of Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR cascades which alter therapy response. Oncotarget 3, 954–987.
McCubrey, J.A., Davis, N.M., Abrams, S.L., Montalto, G., Cervello, M., Basecke, J., Libra, M., Nicoletti, F., Cocco, L., Martelli, A.M., Steelman, L.S., 2014. Diverse roles of GSK-3: tumor promoter-tumor suppressor, target in cancer therapy. Adv. Biol. Regul. 54, 176–196.
McCubrey, J.A., Rakus, D., Gizak, A., Steelman, L.S., Abrams, S.L., Lertpiriyapong, K., Fitzgerald, T.L., Yang, L.V., Montalto, G., Cervello, M., Libra, M., Nicoletti, F., Scalisi, A., Torino, F., Fenga, C., Neri, L.M., Marmiroli, S., Cocco, L., Martelli, A.M., 2016. Effects of mutations in Wnt/β-catenin, hedgehog, Notch and PI3K pathways on GSK-3 activity-Diverse effects on cell growth, metabolism and cancer. Biochim. Biophys. Acta 1863, 2942–2976.
Meijer, L., Skaltsounis, A.L., Magiatis, P., Polychronopoulos, P., Knockaert, M., Leost, M., Ryan, X.P., Vonica, C.A., Brivanlou, A., Dajani, R., Crovace, C., Tarricone, C., Musacchio, A., Roe, S.M., Pearl, L., Greengard, P., 2003. GSK-3-selective inhibitors derived from Tyrian purple indirubins. Chem. Biol. 10, 1255–1266.
Meijer, L.L., Garajova, I., Caparello, C., Le Large, T.Y., Frampton, A., Vasile, E., Funel, N., Kazemier, G., Giovannetti, E., 2020. Plasma miR-181a-5p down-regulation predicts response and improved survival after FOLFIRINOX in pancreatic ductal adenocarcinoma. Ann Surgery. 271, 1137–1147.

Melisi, D., Xia, Q., Paradiso, G., Ling, J., Moccia, T., Carbone, C., Budillon, A., Abbruzzese, J.L., Chiao, P.J., 2011. Modulation of pancreatic cancer chemoresistance by inhibition of TAK1. J. Natl. Cancer Inst. 103, 1190–1204.
Meng, Q., Shi, S., Liang, C., et al., 2018. Abrogation of glutathione peroxidase-1 drives EMT and chemoresistance in pancreatic cancer by activating ROS-mediated Akt/GSK3β/Snail signaling. Oncogene 37, 5843–5857.
Miyashita, K., Nakada, M., Shakoori, A., Ishigaki, Y., Shimasaki, T., Motoo, Y., Kawakami, K., Minamoto, T., 2009. An emerging strategy for cancer treatment targeting aberrant glycogen synthase kinase 3 beta. Anticancer Agents Med. Chem. 9, 1114–1122.
Mora, A., Sabio, G., Risco, A.M., Cuenda, A., Alonso, J.C., Soler, G., Centeno, F., 2002. Lithium blocks the PKB and GSK3 dephosphorylation induced by ceramide through protein phosphatase-2A. Cell. Signal. 14, 557–562.
Nagini, S., Sophia, J., Mishra, R., 2019. Glycogen synthase kinases: moonlighting proteins with theranostic potential in cancer. Semin. Cancer Biol. 56, 25–36.
Namba, T., Kodama, R., Moritomo, S., Hoshino, T., Mizushima, T., 2015. Zidovudine, an anti-viral drug, resensitizes gemcitabine-resistant pancreatic cancer cells to gemcitabine by inhibition of the Akt-GSK3β-Snail pathway. Cell Death Dis. 6, e1795.
NCT01287520, 2018. Eli Lilly and Company. Phase 1 Dose Escalation Study of LY2090314 in Patients With Advanced or Metastatic Cancer in Combination With Pemetrexed and Carboplatin. https://clinicaltrials.gov/ct2/show/NCT01287520.
NCT01632306, 2019. Eli Lilly and Company. Phase I/II Study of LY2090314 and Chemotherapy in Metastatic Pancreatic Cancer Patients With Metastases Amenable to Biopsy. https://clinicaltrials.gov/ct2/show/NCT01632306.
NCT03678883, 2021. Actuate Therapeutics Inc. Phase 1/2 Study of 9-ING-41, a Glycogen Synthase Kinase-3 Beta (GSK-3β) Inhibitor, as a Single Agent and Combined With Chemotherapy, in Patients With Refractory Hematologic Malignancies or Solid Tumors. https://clinicaltrials.gov/ct2/show/NCT03678883.
Okamoto, T., Sanda, T., Asamitsu, K., 2007. NF-kappa B signaling and carcinogenesis. Curr. Pharm. Des. 13, 447–462.
Ougolkov, A.V., Fernandez-Zapico, M.E., Savoy, D.N., Urrutia, R.A., Billadeau, D.D., 2005. Glycogen synthase kinase-3beta participates in nuclear factor kappaB-mediated gene transcription and cell survival in pancreatic cancer cells. Cancer Res. 65, 2076–2081.
Ougolkov, A.V., Fernandez-Zapico, M.E., Bilim, V.N., Smyrk, T.C., Chari, S.T., Billadeau, D.D., 2006. Aberrant nuclear accumulation of glycogen synthase kinase-3beta in human pancreatic cancer: association with kinase activity and tumor dedifferentiation. Clin. Cancer Res. 12, 5074–5081.
Pasquali, L., Busceti, C.L., Fulceri, F., Paparelli, A., Fornai, F., 2010. Intracellular pathways underlying the effects of lithium. Behav. Pharmacol. 21, 473–492.
Peng, Z., Ji, Z., Mei, F., Lu, M., Ou, Y., Cheng, X., 2013. Lithium inhibits tumorigenic potential of PDA cells through targeting Hedgehog-GLI signaling pathway. PLoS One 8, e61457.
Phiel, C.J., Klein, P.S., 2001. Molecular targets of lithium action. Annu. Rev. Pharmacol. Toxicol. 41, 789–813.
Pusceddu, S., Ghidini, M., Torchio, M., Corti, F., Tomasello, G., Niger, M., Prinzi, N., Nichetti, F., Coinu, A., Di Bartolomeo, M., Cabiddu, M., Passalacqua, R., de Braud, F., Petrelli, F., 2019. Comparative effectiveness of gemcitabine plus nab-paclitaxel and FOLFIRINOX in the first-line setting of metastatic pancreatic cancer: a systematic review and meta-analysis. Cancers 11.
Rahib, L., Smith, B.D., Aizenberg, R., Rosenzweig, A.B., Fleshman, J.M., Matrisian, L.M., 2014. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res. 74, 2913–2921.
Randazzo, O., Papini, F., Mantini, G., Gregori, A., Parrino, B., Liu, D.S.K., Cascioferro, S., Carbone, D., Peters, G.J., Frampton, A.E., Garajova, I., Giovannetti, E., 2020. "Open Sesame?": impact of biomarker role and molecular mechanisms influencing the human equilibrative nucleoside transporter-1 in the uptake and cytotoxicity of gemcitabine in pancreatic cancer. Cancers 12, 3206.
Riobó, N.A., Lu, K., Ai, X., Haines, G.M., Emerson, C.P., 2006. Phosphoinositide 3-kinase and Akt are essential for Sonic Hedgehog signaling. Proc. Natl. Acad. Sci. U. S. A. 103, 4505–4510.
Rizzieri, D.A., Cooley, S., Odenike, O., Moonan, L., Chow, K.H., Jackson, K., Wang, X., Brail, L., Borthakur, G., 2016. An open-label phase 2 study of glycogen synthase kinase-3 inhibitor LY2090314 in patients with acute leukemia. Leuk. Lymphoma 57, 1800–1806.
Ryves, W.J., Harwood, A.J., 2001. Lithium inhibits glycogen synthase kinase-3 by competition for magnesium. Biochem. Biophys. Res. Commun. 280, 720–725.
Saiki, Y., Yoshino, Y., Fujimura, H., Manabe, T., Kudo, Y., Shimada, M., Mano, N., Nakano, T., Lee, Y., Shimizu, S., Oba, S., Fujiwara, S., Shimizu, H., Chen, N., Nezhad, Z.K., Jin, G., Fukushige, S., Sunamura, M., Ishida, M., Motoi, F., Egawa, S., Unno, M., Horii, A., 2012. DCK is frequently inactivated in acquired gemcitabine-resistant human cancer cells. Biochem. Biophys. Res. Commun. 421, 98–104.
Santoro, R., Zanotto, M., Simionato, F., Zecchetto, C., Merz, V., Cavallini, C., Piro, G., Sabbadini, F., Boschi, F., Scarpa, A., Melisi, D., 2020. Modulating TAK1 expression inhibits YAP and TAZ oncogenic functions in pancreatic cancer. Mol. Cancer Ther. 19, 247–257.
Sarantis, P., Koustas, E., Papadimitropoulou, A., Papavassiliou, A.G., Karamouzis, M.V., 2020. Pancreatic ductal adenocarcinoma: treatment hurdles, tumor microenvironment and immunotherapy. World J. Gastrointest. Oncol. 12, 173–181.
Saraswati, A.P., Ali Hussaini, S.M., Krishna, N.H., Babu, B.N., Kamal, A., 2018. Glycogen synthase kinase-3 and its inhibitors: potential target for various therapeutic conditions. Eur. J. Med. Chem. 144, 843–858.
Seino, T., Kawasaki, S., Shimokawa, M., Tamagawa, H., Toshimitsu, K., Fujii, M., Ohta, Y., Matano, M., Nanki, K., Kawasaki, K., Takahashi, S., Sugimoto, S., Iwasaki, E., Takagi, J., Itoi, T., Kitago, M., Kitagawa, Y., Kanai, T., Sato, T., 2018. Human pancreatic tumor organoids reveal loss of stem cell niche factor dependence during disease progression. Cell Stem Cell 22, 454-467.e6.
Shimasaki, T., Ishigaki, Y., Nakamura, Y., Takata, T., Nakaya, N., Nakajima, H., Sato, I., Zhao, X., Kitano, A., Kawakami, K., Tanaka, T., Takegami, T., Tomosugi, N., Minamoto, T., Motoo, Y., 2012. Glycogen synthase kinase 3β inhibition sensitizes pancreatic cancer cells to gemcitabine. J. Gastroenterol. 47, 321–333.
Shin, S., Wolgamott, L., Yu, Y., Blenis, J., Yoon, S.-O., 2011. Glycogen synthase kinase (GSK)-3 promotes p70 ribosomal protein S6 kinase (p70S6K) activity and cell proliferation. Proc. Natl. Acad. Sci. U. S. A. 108, E1204–1213.
Shukla, S.K., Purohit, V., Mehla, K., Gunda, V., Chaika, N.V., Vernucci, E., King, R.J., Abrego, J., Goode, G.D., Dasgupta, A., Illies, A.L., Gebregiworgis, T., Dai, B., Augustine, J.J., Murthy, D., Attri, K.S., Mashadova, O., Grandgenett, P.M., Powers, R., Ly, Q.P., Lazenby, A.J., Grem, J.L., Yu, F., Matés, J.M., Asara, J.M., Kim, J.W., Hankins, J.H., Weekes, C., Hollingsworth, M.A., Serkova, N.J., Sasson, A. R., Fleming, J.B., Oliveto, J.M., Lyssiotis, C.A., Cantley, L.C., Berim, L., Singh, P.K., 2017. MUC1 and HIF-1alpha signaling crosstalk induces anabolic glucose metabolism to impart gemcitabine resistance to pancreatic cancer. Cancer Cell 32, 71-87.e7.
Siegel, R.L., Miller, K.D., Fuchs, H.E., Jemal, A., 2021. Cancer statistics, 2021. CA Cancer J. Clin. 71, 7–33.
Singh, T.J., Zaidi, T., Grundke-Iqbal, I., Iqbal, K., 1995. Modulation of GSK-3-catalyzed phosphorylation of microtubule-associated protein tau by non-proline-dependent protein kinases. FEBS Lett. 358, 4–8.
Song, E.Y., Palladinetti, P., Klamer, G., Ko, K.H., Lindeman, R., O'Brien, T.A., Dolnikov, A., 2010. Glycogen synthase kinase–3β inhibitors suppress leukemia cell growth. Exp. Hematol. 38 (908-921) e1.
Stamos, J.L., Chu, M.L.-H., Enos, M.D., Shah, N., Weis, W.I., 2014. Structural basis of GSK-3 inhibition by N-terminal phosphorylation and by the Wnt receptor LRP6. eLife 3, e01998.
Sun, A., Li, C., Chen, R., Huang, Y., Chen, Q., Cui, X., Liu, H., Thrasher, J.B., Li, B., 2016. GSK-3β controls autophagy by modulating LKB1-AMPK pathway in prostate cancer cells. Prostate 76, 172–183.
Supadmanaba, I.G.P., Comandatore, A., Morelli, L., Giovannetti, E., Lagerweij, T., 2021. Organotypic-liver slide culture systems to explore the role of extracellular vesicles in pancreatic cancer metastatic behavior and guide new therapeutic approaches. Expert Opin. Drug Metab. Toxicol. 21, 1–10.
Sutherland, C., 2011. What are the bona fide GSK3 substrates? Int. J. Alzheimers Dis. 2011, 505607.
ter Haar, E., Coll, J.T., Austen, D.A., Hsiao, H.M., Swenson, L., Jain, J., 2001. Structure of GSK3beta reveals a primed phosphorylation mechanism. Nat. Struct. Biol. 8, 593–596.
Tran, F.H., Zheng, J.J., 2017. Modulating the wnt signaling pathway with small molecules. Protein Sci. Publ. Protein Soc. 26, 650–661.
Uehara, M., Domoto, T., Takenaka, S., Bolidong, D., Takeuchi, O., Miyashita, T., Minamoto, T., 2020. Glycogen synthase kinase-3β participates in acquired resistance to gemcitabine in pancreatic cancer. Cancer Sci. 111, 4405–4416.
Vulpetti, A., Crivori, P., Cameron, A., Bertrand, J., Brasca, M.G., D'Alessio, R., Pevarello, P., 2005. Structure-based approaches to improve selectivity: CDK2-GSK3beta binding site analysis. J. Chem. Inf. Model. 45, 1282–1290.
Walz, A., Ugolkov, A., Chandra, S., Kozikowski, A., Carneiro, B.A., O'Halloran, T.V., Giles, F.J., Billadeau, D.D., Mazar, A.P., 2017. Molecular pathways: revisiting glycogen synthase Kinase-3β as a target for the treatment of cancer. Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res. 23, 1891–1897.
Waters, A.M., Channing, D.J., 2018. KRAS: the critical driver and therapeutic target for pancreatic cancer. Cold SpringHarb. Perspect. Med. 8, a031435.
Webster, M.T., Rozycka, M., Sara, E., Davis, E., Smalley, M., Young, N., Dale, T.C., Wooster, R., 2000. Sequence variants of the axin gene in breast, colon, and other cancers: an analysis of mutations that interfere with GSK3 binding. Genes Chromosomes Cancer 28, 443–453.
Wilson, W., Baldwin, A.S., 2008. Maintenance of constitutive IkappaB kinase activity by glycogen synthase kinase-3alpha/beta in pancreatic cancer. Cancer Res. 68, 8156–8163.
Woodgett, J.R., 1990. Molecular cloning and expression of glycogen synthase kinase-3/factor A. EMBO J. 9, 2431–2438.
Wu, D., Pan, W., 2010. GSK3: a multifaceted kinase in Wnt signaling. Trends Biochem. Sci. 35, 161–168.
Xia, Y., Shen, S., Verma, I.M., 2014. NF-κB, an active player in human cancers. Cancer Immunol. Res. 2, 823–830.
Xie, S., Wang, C., 2017. GSK-3 inhibition suppresses lung cancer cell survival, metastasis and proliferation through down-regulation the phosphorylation sites of CAP1. Eur. Respir. J. 50.
Xu, C., Kim, N.-G., Gumbiner, B.M., 2009. Regulation of protein stability by GSK3 mediated phosphorylation. Cell Cycle Georget. Tex 8, 4032–4039.
Yang, S., Kimmelman, A.C., 2011. A critical role for autophagy in pancreatic cancer. Autophagy 7, 912–913.
Yang, S., Wang, X., Contino, G., Liesa, M., Sahin, E., Ying, H., Bause, A., Li, Y., Stommel, J.M., Dell'antonio, G., Mautner, J., Tonon, G., Haigis, M., Shirihai, O.S., Doglioni, C., Bardeesy, N., Kimmelman, A.C., 2011. Pancreatic cancers require autophagy for tumor growth. Genes Dev. 25, 717–729.
Yoneyama, H., Takizawa-Hashimoto, A., Takeuchi, O., Watanabe, Y., Atsuda, K., Asanuma, F., Yamada, Y., Suzuki, Y., 2015. Acquired resistance to gemcitabine and cross-resistance in human pancreatic cancer clones. Anticancer Drugs 26, 90–100.
Yoshino, Y., Ishioka, C., 2015. Inhibition of glycogen synthase kinase-3 beta induces apoptosis and mitotic catastrophe by disrupting centrosome regulation in cancer cells. Sci. Rep. 5, 13249.

Yu, M., Ting, D.T., Stott, S.L., et al., 2012. RNA sequencing of pancreatic circulating tumour cells implicates WNT signalling in metastasis. Nature 487, 510–513.
Zamek-Gliszczynski, M.J., Abraham, T.L., Alberts, J.J., Kulanthaivel, P., Jackson, K.A., Chow, K.H., McCann, D.J., Hu, H., Anderson, S., Furr, N.A., Barbuch, R.J., Cassidy, K.C., 2013. Pharmacokinetics, metabolism, and excretion of the glycogen synthase kinase-3 inhibitor LY2090314 in rats, dogs, and humans: a case study in rapid clearance by extensive metabolism with low circulating metabolite exposure. Drug Metab. Dispos. Biol. Fate Chem. 41, 714–726.
Zeng, S., Pöttler, M., Lan, B., Grützmann, R., Pilarsky, C., Yang, H., 2019. Chemoresistance in pancreatic cancer. Int. J. Mol. Sci. 20.
Zhang, F., Phiel, C.J., Spece, L., Gurvich, N., Klein, P.S., 2003. Inhibitory phosphorylation of glycogen synthase kinase-3 (GSK-3) in response to lithium. Evidence for autoregulation of GSK-3. J. Biol. Chem. 278, 33067–33077.
Zhang, J.-S., Koenig, A., Harrison, A., Ugolkov, A.V., Fernandez-Zapico, M.E., Couch, F. J., Billadeau, D.D., 2011. Mutant K-Ras increases GSK-3β gene expression via an ETS-p300 transcriptional complex in pancreatic cancer. Oncogene 30, 3705–3715.
Zhang, J.-S., Herreros-Villanueva, M., Koenig, A., de Narvajas, A.A., Gomez, T.S., Meng, X., Bujanda, L., Ellenrieder, V., Li, X.K., Kaufmann, S.H., Billadeau, D.D., 2014. Differential activity of GSK-3 isoforms regulates NF-κB and TRAIL- or TNFα induced apoptosis in pancreatic cancer cells. Cell Death Dis. 5, e1142.
Zhang, X., Castanotto, D., Nam, S., Horne, D., Stein, C., 2017. 6BIO enhances oligonucleotide activity in cells: a potential combinatorial anti-androgen receptor therapy in prostate cancer cells. Mol. Ther. 25, 79–91.
Zhitomirsky, B., Assaraf, Y.G., 2015. Lysosomal sequestration of hydrophobic weak base chemotherapeutics triggers lysosomal biogenesis and lysosome-dependent cancer multidrug resistance. Oncotarget 6 (January (2)), 1143–1156. https://doi.org/10.18632/oncotarget.2732.
Zhitomirsky, B., Assaraf, Y.G., 2016. Lysosomes as mediators of drug resistance in cancer. Drug Resist. Updat. 24 (January), 23–33.
Zhitomirsky, B., Yunaev, A., Kreiserman, R., Kaplan, A., Stark, M., Assaraf, Y.G., 2018. Lysosomotropic drugs activate TFEB via lysosomal membrane fluidization and consequent inhibition of mTORC1 activity. Cell Death Dis. 9 (December (12)), 1191.
Zhou, B., Xu, J.W., Cheng, Y.G., Gao, J.Y., Hu, S.Y., Wang, L., Zhan, H.X., 2017. Early detection of pancreatic cancer: where are we now and where are we going? Int. J. Cancer 141, 231–241.
Zhu, H., Lo, H.-W., 2010. The human glioma-associated oncogene homolog 1 (GLI1) family of transcription factors in gene regulation and diseases. Curr. Genomics 11, 238–245.