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Targeting GSK-3: a promising approach for cancer therapy?

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Glycogen synthase kinase (GSK)-3 has emerged as one of the most attractive therapeutic targets for the treatment of multiple neurological diseases, including Alzheimer's, stroke and bipolar disorders, as well as noninsulin-dependent diabetes mellitus and inflammation. Although the prominent role of GSK-3 in the adenomatous polyposis coli (APC)–β-catenin destruction complex implies that inhibition of GSK-3 could possibly lead to tumor promotion through the activation of β-catenin, several recent studies have shed new light on the activity of GSK-3 in cancer and provide insight into the molecular mechanisms by which it regulates tumor cell proliferation and survival of multiple human malignancies. In fact, GSK-3β is a critical regulator of nuclear factor (NF)κB nuclear activity, suggesting that inhibition of GSK-3β could be effective in the treatment of a wide variety of tumors with constitutively active NFκB. Herein, the authors will discuss the current understanding of the role of GSK-3 in human cancer and its potential as a therapeutic target.

GSK-3 & human diseases

The cytoplasmic serine/threonine protein kinase glycogen synthase kinase (GSK)-3 was first described as a component of the metabolic pathway for glycogen synthase regulation that is sensitive to insulin-mediated inhibition [1]. There are two homologous mammalian isoforms encoded by different genes, GSK-3α and GSK-3β [2]. Recently, GSK-3 has emerged as a potential therapeutic target in various diseases, in which its overexpression is linked to pathology (e.g., Alzheimer's disease and noninsulin-dependent diabetes mellitus [NIDDM]). Multiple studies link GSK-3 to Alzheimer's disease, owing to the fact that the microtubule-binding protein tau is hyperphosphorylated by GSK-3, leading to the formation of paired-helical filaments that characterize Alzheimer's disease and other dementias [3]. Apart from a role in neurological pathologies, GSK-3 participates in the development of NIDDM, a disease often associated with chronic inhibition of muscle glycogen synthase [4]. In NIDDM, GSK-3 inhibitors mimic the insulin-activated signaling pathway by activating glycogen synthesis through the dephosphorylation and activation of glycogen synthase [4,5]. In addition, it has also been suggested that GSK-3 might participate in inflammatory processes by positively regulating nuclear factor (NF)κB transcriptional activity, since administration of a GSK-3 inhibitor potently suppressed proinflammatory responses and mediated protection from endotoxin shock in mice [6]. Lastly, inhibition of GSK-3 was found to be useful in the therapy of organ dysfunction associated with sepsis, shock and other diseases associated with local or systemic inflammation [7]. Taken together, these recent observations identify GSK-3 as a potential therapeutic target in multiple human pathological processes.

GSK-3 & cancer: a paradigm with a controversial history

GSK-3 is purported to participate in neoplastic transformation and tumor development based on its role in the canonical wingless-type (Wnt)–β-catenin signaling pathway. In Wnt signaling, GSK-3 is a critical component of the adenomatous polyposis coli (APC)–β-catenin destruction complex [8]. When β-catenin is phosphorylated by GSK-3, it becomes a target for ubiquitin-mediated proteosomal degradation, thereby limiting the amount of free β-catenin that can accumulate in the nucleus, where it can function as a transcriptional coactivator for the T-cell factor (TCF)/lymphoid enhancer factor (LEF) transcription factors to drive the expression of a certain set of genes, including oncogenes (e.g., *cyclin D1*, matrix metalloproteinase [*MMP*]-*7*) [8]. Exposure of cells to Wnt ligands leads to inactivation of GSK-3 through an as yet unclear mechanism. Of note, tissues from mice lacking GSK-3β do not show evidence of accumulated β-catenin, even though total GSK-3 levels are reduced by 50% and there is zero cellular GSK-3β [9]. Immunoprecipitation of axin from these tissues reveals that GSK-3β is simply replaced by GSK-3α (in wild-type cells, both GSK-3α and GSK-3β are found bound to axin), indicating that these two kinases can substitute for each other in

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the regulation of β-catenin [2]. A critical aspect of GSK function in the Wnt pathway is that GSK-3 appears to be insulated from regulators of GSK-3 that lie outside of the Wnt pathway [2]. For example, insulin/Akt signaling leads to inhibition of GSK-3 via serine phosphorylation (Ser21 in GSK-3α and Ser9 in GSK-3β) but does not cause accumulation of β-catenin. Conversely, Wnt signaling does not affect insulin/Akt signaling and does not lead to serine phosphorylation of either GSK-3 isoform [10]. How this insulation occurs is unclear, but it probably stems from the effective sequestration of a fraction of GSK-3 with axin in the destruction complex [2]. It is of significant interest that embryonic stem cells lacking GSK-3 (α and β), but expressing a constitutive active version of GSK3-α (S21A), can still regulate β-catenin nuclear accumulation in response to Wnt signaling [11]. Thus, these data would suggest that Wnt signaling does not inactivate GSK-3, but more likely disrupts the formation of the β-catenin destruction complex.

Akt is frequently activated in human cancer, including carcinomas, glioblastoma multiforme and various hematological malignancies [12]. Although activated Akt inhibits GSK-3 through the phosphorylation of GSK-3 at Ser21/Ser9 (which reflects the physiological mechanism of insulin action), this inactivation does not affect β-catenin levels in the cell and does not mean that GSK-3 is inhibited completely in human cancer. The best example of this are two pancreatic cancer cell lines, PANC1 and ASPC1, which exhibit 30- and 50-fold amplification of AKT2, respectively, and high levels of *AKT2* RNA and protein [13]. However, using an *in vitro* kinase assay, the authors have recently demonstrated that GSK-3β is highly active in PANC1 and ASPC1 cancer cell lines, suggesting that although some pools of GSK-3 can be phosphorylated by Akt at Ser21/Ser9 and inhibited, certain pools of GSK-3 remain active in cancer cells [14]. Moreover, another study demonstrated high activity and increased expression level of GSK-3β in colon cancer cell lines and in human colorectal carcinomas by *in vitro* kinase assay and Western blotting [15]. Significantly, this study demonstrated high levels of active phospho-Akt Ser473 in 10 out of 20 human colorectal carcinomas, but lower levels of inactive phospho-GSK-3β Ser9 were found in nine of these ten tumors (expressing active Akt) than in their normal counterparts [15]. Additionally, compared with normal tissue, GSK-3β was overexpressed in most colon cancer tumor samples [15]. These studies suggest that Akt activation and GSK-3 inhibitory phosphorylation are not always correlated in human tumors *in vivo* and a certain pool of GSK-3 remains active in cancer cells irrespective of Akt activation. Thus, while GSK-3 has been shown to negatively regulate the stability and expression of cell-cycle regulators, such as cyclin D1, cyclin E, c-Jun and c-Myc, which are linked with tumorigenesis [16], GSK-3 may be involved in cancer cell proliferation and survival.

Taken together, one would predict that GSK-3 is a putative tumor-suppressor protein, since its inhibition is expected to mimic the activation of the Wnt-signaling pathway and stabilize oncogenic proteins. Thus, anti-GSK-3 therapy raises concerns that inhibition of GSK-3 could presumably lead to tumorigenesis. However, to date there have been no reports of GSK-3 mutations in human cancer and, in fact, Wnt signaling, cyclin D1 levels and β-catenin nuclear accumulation are not perturbed in GSK-3β-deficient mice [9]. Moreover, the authors' studies suggest that, even in colon cancer, where β-catenin dysregulation is involved in the pathogenesis of the tumor, GSK-3β remains active, is overexpressed in the cancer cells and is required for colon cancer cell proliferation and survival [15]. Moreover, 60 days of lithium treatment, a potent inhibitor of GSK-3 with a limited degree of inhibition, in APC mutant mice, did not produce a significant increase in the number of tumors in these genetically predisposed mice [17]. In addition, it was reported that the risk of cancer development in psychiatric patients treated with lithium carbonate is significantly lower than in the general population, and that an inverse relationship has been observed between cancer morbidity and lithium dosage [18]. However, it is unclear whether the concentration of lithium administered in these cases would be able to inactivate GSK-3 sufficiently, in order to stabilize β-catenin and permit its nuclear accumulation and transactivation. Yet, lithium treatment was found to lead to decreased levels of cyclin E and protein kinase B (PKB)/Akt and inhibition of proliferation in hepatocellular carcinoma cell lines [19]. Thus, the role of GSK-3 in human cancer remains enigmatic and controversial. On the one hand, GSK-3 should function as a tumor suppressor by regulating the activation of β-catenin signaling, but on the other hand, GSK-3 inhibition does not increase the incidence of cancer and seems to correlate with decreased tumor cell proliferation and survival.

Inhibition of GSK-3β suppresses cancer cell proliferation & survival by abrogating NFκB nuclear activity

The activation of NFκB is a double-edged sword. While needed for proper immune system function, inappropriate NFκB activation can mediate inflammation and tumorigenesis. In fact, constitutively active NFκB has been identified not only in cancer cell lines, but also in human tumor tissues derived from patients with multiple myeloma [20], acute myelogenous leukemia [21], acute lymphocyte leukemia [22], chronic myelogenous leukemia [23], prostate [24], breast [25] and pancreatic [26] cancers. A major consequence of tumors that have constitutive NFκB activity is their increased proliferation through the upregulation of cyclin D1, and their resistance to chemotherapy and radiation through the increased expression of survival genes (e.g., *Bcl-2*, *Bcl-X_L*, *XIAP*). In fact, NFκB activation is part of the cancer cell's autodefense mechanism, since most chemotherapeutic agents and radiation that induce apoptosis also activate NFκB [27], and thus hyperactive NFκB signaling can contribute to tumor chemoresistance and radioresistance [28]. In addition, NFκB participates in tumor neovascularization, invasion and metastasis through the upregulation of vascular endothelial growth factor (VEGF) and MMP-9 [29,30]. Consistent with these roles of NFκB in cancer, suppression of NFκB in tumors inhibits its metastasis, causes cell-cycle arrest and leads to apoptosis [30,31]. Together, all of these studies highlight the crucial role of NFκB in tumorigenesis, suggesting NFκB as an attractive target in the treatment of cancer.

Surprisingly, similar to the disruption of the *NFκB* p65 or *IKK*β genes, ablation of the murine *GSK-3*β gene resulted in embryonic lethality due to hepatocyte apoptosis and massive liver degeneration [9,32,33]. These findings pointed to an unexpected role for GSK-3β (but not GSK-3α) in the mechanism of NFκB activation and suggest that GSK-3β may be a potential therapeutic target in human cancer. In fact, the authors have shown that pharmacological inhibition of GSK-3 or genetic depletion of GSK-3β by RNA interference (RNAi) suppresses basal NFκB transcriptional activation of a subset of anti-apoptotic (*XIAP*, *Bcl-2*) and proliferation (*cyclin D1*) genes, leading to decreased pancreatic cancer cell proliferation and survival [14]. Moreover, pharmacological inhibition of GSK-3 arrests pancreatic tumor growth *in vivo* and leads to suppression of NFκB transcriptional activity and decreased pancreatic cancer cell proliferation and survival in established tumor xenografts [AV Ougolkov and DD Billadeau, Unpublished Data]. In addition, it has been demonstrated that lithium-induced strong growth inhibition in 9 out of 12 hepatocellular carcinoma cell lines [19] and lithium enhanced the *in vivo* antitumor action of tumor necrosis factor (TNF) [34]. Moreover, GSK-3 inhibition was also shown to be useful in treating prostate cancer, with the inhibition of GSK-3 reducing cancer cell proliferation and dramatically enhancing TNF-related apoptosis inducing ligand (TRAIL)-induced apoptosis in prostate cancer cell lines [35,36]. Lastly, pharmacological inhibition of GSK-3 or depletion of GSK-3β by RNAi induced apoptosis and attenuated proliferation of colon cancer cells [15]. Thus, in these examples, inhibition of GSK-3 using small molecule inhibitors, or RNAi to specifically deplete GSK-3β results in decreased NFκB activity in cancer cells and an increase in sensitivity to compounds that induce apoptosis.

Recently, it has been demonstrated that ablation of GSK-3β in HCT116 $p53^{+/+}$ colon cancer cells activates p53-dependent apoptosis and antagonizes xenograft tumor growth *in vivo* [37]. In fact, NFκB is constitutively active in HCT116 colon cancer cells [38] and *Twist*, an NFκB-regulated target gene, can inhibit p53-induced apoptosis, possibly by interfering with the ARF–MDM pathway [39]. NFκB itself can also upregulate MDM2 and suppress p53 stabilization [40]. Thus, depletion of GSK-3β may induce p53-dependent apoptosis through inhibition of basal NFκB activity in HCT116 $p53^{+/+}$ colon cancer cells, although other mechanisms cannot be excluded. In addition, several cancer cell lines that are mutant for p53, including the colon cancer cell line SW480 and the pancreatic cancer cell lines MIA-PaCa2 and BXPC3, undergo apoptosis following incubation with GSK-3 inhibitors or depletion of GSK-3β by RNAi [14,15]. Thus, there may be p53-dependent, as well as p53-independent, mechanisms controlling apoptosis following the inhibition of GSK-3. Whether this is cell-type specific or owing to the genetic make-up of the tumor cells is unclear and will require further study.

The exact mechanism by which GSK-3β impacts NFκB activity is unknown. Using GSK-3β-deficient mouse embryonic fibroblasts (MEFs), it has been demonstrated that the early steps leading to NFκB activation following

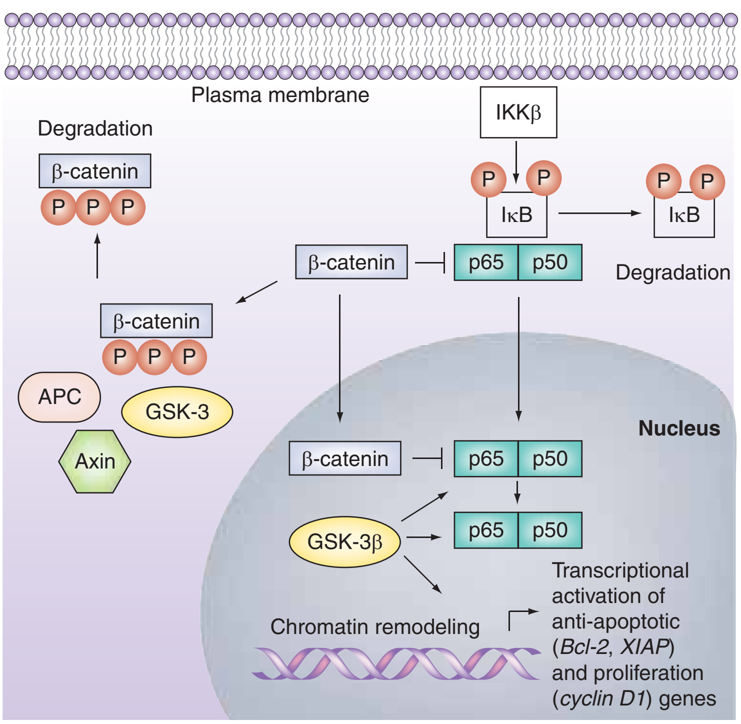
TNFα treatment (degradation of inhibitor of κB [IκB]α and translocation of NFκB to the nucleus) were unaffected by the loss of GSK-3β, indicating that NFκB is regulated by GSK-3β at the level of the transcriptional complex [9]. Consistent with this idea, the authors have shown that GSK-3β influences NFκB-mediated gene transcription in pancreatic cancer cells at a point distal to the IκB kinase [IKK] complex, as only ectopic expression of the NFκB subunits p65/p50, but not an IKKβ constitutively active mutant, could rescue the decreased cellular proliferation and survival associated with GSK-3β inhibition [14]. These data rule out an effect of GSK-3β on the cascade of proteins that culminates in phosphorylation of IκBα and its degradation, and suggest that GSK-3β must be regulating the nuclear activity of NFκB p65/p50 (Figure 1). It is of interest, that GSK-3β is found colocalized with NFκB p65 in the nuclei of pancreatic cancer cell lines and samples from pancreatic cancer patients [AV Ougolkov and DD Billadeau, Unpublished Data].

Recently, two reports demonstrated that increased levels of β-catenin can antagonize NFκB activity [41,42], suggesting that the inhibition of GSK-3 (both GSK-3α and GSK-3β) may affect NFκB activity through the stabilization of β-catenin–p65 complexes that are transcriptionally inactive (Figure 1). However, β-catenin levels are unaffected in GSK-3β null fibroblasts, suggesting the possibility of an effect on NFκB by GSK-3β that is independent of β-catenin [9]. Consistent with a role for GSK-3β in regulating the nuclear activity of p65, a recent study demonstrated that inhibition of GSK-3β leads to decreased TNFα-induced binding of NFκB to the promoters of a subset of target genes in fibroblasts and intestinal epithelial cells [43]. Consistent with this observation, using chromatin immunoprecipitation, the authors have found that p65 is no longer bound to the promoters of several target genes following GSK-3 inhibition in pancreatic cancer cell lines [AV Ougolkov and DD Billadeau, Unpublished Data]. Together, these data suggest that GSK-3 regulates the nuclear activity of NFκB, but the mechanisms involved have not yet been fully elucidated. It is of interest that GSK-3β is able to phosphorylate several sites in the transactivation domain of p65 and in this way may regulate the binding of p65 to the promoters of its target genes. In fact, the loss of GSK-3β-mediated phosphorylation of p65 results in TNFα sensitivity in hepatocytes owing to greatly diminished NFκB transcriptional activity [44], whereas another study demonstrated that phosphorylation of p65 at Ser468 by GSK-3β negatively regulates basal NFκB activity [45]. Supporting the latter hypothesis, another study suggests that GSK-3β can have a repressive effect on NFκB activity [46]. Although it is possible that GSK-3β controls NFκB nuclear activity through direct phosphorylation of NFκB p65, with p65 modification affecting DNA binding activity or dimerization [44], it is also possible that GSK-3β may affect the chromatin structure, thereby facilitating accessibility of transcription factors, such as NFκB, at the promoter regions of target genes (Figure 1). Recently, the authors have found that GSK-3 regulates histone H3 modification at the promoters of a subset of NFκB-mediated genes in pancreatic cancer cells, and through this, GSK-3 may contribute to p65/p50 binding to the promoters of its target genes [AV Ougolkov and DD Billadeau, Unpublished Data]. Thus, it is possible, that GSK-3β may affect the regulation of NFκB at several levels but the exact mechanism(s) by which it exerts its effects on NFκB will require further study.

GSK-3 & carbohydrate metabolism in cancer

In addition to GSK-3β affecting NFκB-mediated cancer cell survival and proliferation, GSK-3 regulates a wide range of cellular processes including carbohydrate metabolism and translation. More than seven decades ago, the German biochemist Otto Warburg made the seminal observation that most tumors relied on anaerobic glycolysis even in the presence of abundant oxygen, a phenomenon now referred to as the Warburg effect [47]. In fact, tumor cells are notorious for their consumption of glucose, a requirement to sustain their high proliferative rate and increased need for macromolecular synthesis. A well known characteristic of malignant cells is their ability to compete for glucose up to 50-times more intensely than the surrounding normal tissue [47,48]. This characteristic of tumor cells has been confirmed and exploited by clinical radiologists using 2-fluoro-2-deoxy-D-glucose-positron emission tomography (PET) imaging. This switch from aerobic to anaerobic metabolism, while not being at all energy efficient (anaerobic glycolysis only realizes two ATP molecules per glucose molecule, whereas an additional 36 molecules could be generated during aerobic oxidative phosphorylation by oxidizing pyruvate to HCO_3), is compensated by an increase in glucose uptake by tumor cells [49]. Interestingly,

Figure 1. Protein cascade highlighting regulation of NFκB nuclear activity.



GSK-3β is required for NFκB nuclear activity, independent of IκB degradation and NFκB nuclear translocation. See text for details.
APC: Adenomatous polyposis coli; GSK: Glycogen synthase kinase; IκB: Inhibitor of κB; IKK: IκB kinase; NF: Nuclear factor.

while the glycolytic phenotype would appear to confer a significant competitive disadvantage on the tumor cells, recent mathematical modeling of the tumor–host interface suggests that the switch to anaerobic metabolism actually favors tumor cell invasion into the surrounding normal parenchyma where the tumor can outcompete normal cells for available resources [50]. Moreover, the anaerobic glycolytic phenotype decreases the extracellular pH surrounding the tumor (owing to the conversion of pyruvate to lactate by lactate dehydrogenase and increased excretion of protons through upregulated Na^+/H^+ antiport and other membrane transporters), resulting in increased p53-dependent apoptosis of normal cells (most tumor cells are p53 mutant and can survive in such a low pH environment), enhanced angiogenesis, decreased immune cell responses, degradation of the interstitial matrix and loss of intercellular gap junctions. Tumor cells also use glucose for intracellular anabolic processes, mainly the synthesis of nucleotides and other macromolecules, which are critical for cell division and proliferation. Taken together, all of these events contribute to and favor tumor growth, invasion and metastasis.

As indicated previously, GSK-3 was discovered initially as an inhibitor of glycogen synthase, which is the rate-limiting kinase in glycogen synthesis. Glycogen, the storage form of carbohydrate in the human body, is found chiefly in the liver and muscle, but can also be stored in other cell types. It is of interest that expression of glycogen is significantly decreased in poorly differentiated cancer cells, which are the most aggressive and demonstrate the highest proliferation rate [51,52]. It is likely that rapidly dividing cancer cells require a constant and high supply of glucose with no need for synthesis and storage of glycogen. By inhibiting glycogen synthesis, GSK-3 may contribute to cancer cell proliferation through complete utilization of glucose for energy needs and macromolecular synthesis. Given the observation that energy metabolism of cancer cells relies mainly on glucose, cancer cells should be more sensitive to alterations in glucose metabolism compared with normal cells. Inhibition of GSK-3 may affect glucose metabolism in cancer cells by upregulating glycogen synthesis leading to alterations in catabolic (anaerobic glycolysis) and anabolic (nucleotides synthesis) processes in the cancer cell, which may result in decreased cancer cell proliferation and survival, although this hypothesis needs to be investigated.

Expression & localization of GSK-3β in cancer cells

GSK-3β protein overexpression has been found in human ovarian [53], colon [15] and pancreatic [AV Ougolkov and DD Billadeau, Unpublished Data] carcinomas. Of note, the study using a mouse hepatic carcinogenesis model demonstrated higher levels of GSK-3β expression in liver tumors than in normal liver tissue, as shown by Western blotting [54]. Although the localization of GSK-3β has not been investigated in human cancer, the authors have found that cytoplasmic overexpression of GSK-3β (normal ductal and acinar cells showed only weak GSK-3β cytoplasmic staining and thus this staining served as an internal control) was observed in most high-grade pancreatic intraepithelial neoplasias (PanIN) lesions and differentiated pancreatic adenocarcinomas, whereas nuclear accumulation of GSK-3β was significantly associated with poorly differentiated pancreatic adenocarcinomas [AV Ougolkov and DD Billadeau, Unpublished Data]. Although GSK-3β does not contain any identifiable nuclear localization or nuclear export signal sequences, it is known to shuttle from the cytoplasm to the nucleus, where it is thought to participate in the regulation of gene transcription through the phosphorylation of transcription factors (e.g., nuclear factor of activated T cells [NFAT], c-Jun) [55,56]. Although the GSK-3β–interacting protein Frat1 has been suggested to bind to GSK-3β in the nucleus and shuttle it to the cytoplasm, the mechanism by which GSK-3β is transported into the nucleus is not clear. However, a recent report has identified the TNF-like family member TNF weak inducer of apoptosis (TWEAK) as a molecule that interacts with GSK-3β [57]. It was found that a membrane cleaved form of TWEAK (sTWEAK), could enter cells that lack its known receptor (Fn14) and promote the nuclear accumulation of GSK-3β and subsequently NFκB-mediated gene transcription, thereby providing a possible link between GSK-3β nuclear accumulation and the regulation of nuclear NFκB activation [57]. It is of interest that many tumor cell lines and tumors express TWEAK. However, the role of TWEAK in the pathogenesis of human malignancies is controversial, since TWEAK can have both proliferative and antiproliferative effects on cancer cell lines. It remains to be determined if TWEAK participates in the nuclear accumulation of GSK-3β in cancer cells, where it could increase basal NFκB activity.

Inhibition of GSK-3 by cyclin-dependent kinase inhibitors: side or major anticancer effect?

As demonstrated in numerous publications, cyclin-dependent kinase (CDK) inhibitors suppress the proliferation and survival of a broad range of cancer cell lines *in vitro* and *in vivo* [58]. In fact, CDK inhibitors, induce cell cycle arrest and apoptosis in cell lines that lack p53 and Rb, where normal cell cycle checkpoints are not in place. Presently, the mechanism by which CDK inhibitors induce apoptosis in cancer cells is unknown, but several studies have convincingly suggested that CDK inhibitors act on targets other than CDKs to induce apoptosis [58]. CDK inhibitors target a variety of kinases and of interest, most reported CDK inhibitors are also powerful GSK-3 inhibitors [59]. For example, staurosporine, its derivative UCN-01 (7-hydroxystaurosporine) and flavopiridol inhibit GSK-3 with an IC_{50} of 15 nM, 70 nM and 450 nM, respectively [59]. Indeed, flavopiridol inhibits TNFα-mediated NFκB activation and induces apoptosis in small cell lung cancer (SCLC) cell lines, but the reason for this effect of flavopiridol is unknown [60]. However, the potential explanation could be found in another study showing that inhibition of GSK-3 suppressed NFκB activity and sensitized hepatocytes toward TNFα-mediated apoptosis [44]. Moreover, similar to genetic depletion of GSK-3β by RNAi [14], CDK inhibitors downregulate expression of NFκB target genes cyclin D1, Bcl-2 and XIAP in cancer cells [61,62]. Thus, it is possible that CDK inhibitors induce apoptosis through inhibition of GSK-3 resulting in decreased NFκB transcriptional activity in cancer cells.

Another question is how CDK inhibitors could be effective in the treatment of small cell lung cancer if these cancer cells are nearly universally Rb negative [63]. Nevertheless, staurosporine suppresses proliferation and survival of SCLC cancer cells [64], which is further evidence of CDK inhibitors off-targeting. Again, this effect could be explained by inhibition of GSK-3 with subsequent inactivation of NFκB-mediated transcription resulting in decreased cancer cell proliferation and survival in SCLC. In fact, the authors have found that inhibition of GSK-3 suppresses NFκB transcriptional activity in SCLC leading to decreased cancer cell proliferation and apoptosis [AV Ougolkov and DD Billadeau, Unpublished Data]. In addition, similarity was observed in the induction of TRAIL-mediated apoptosis using either flavopiridol [60] or GSK-3 inhibitors [36] in cancer cells. Of course, although some of these suggestions are highly speculative, previously published papers on the cellular effects of CDK inhibitors need to be re-evaluated in terms of GSK-3 inhibition and their effects on cancer cell proliferation and survival.

Future perspective

As our knowledge of the function and regulation of GSK-3 in cancer progresses, we become aware of the potential benefits of inhibiting this kinase to treat this disease. However, several questions remain to be answered: what is the expression profile and subcellular localization of GSK-3 in different types of human cancer? What drives the nuclear accumulation of GSK-3β in cancer cells? How does GSK-3β potentiate NFκB activity in the cancer cell nucleus? Does GSK-3β regulate other transcription factors that modulate tumor cell proliferation and survival? Is GSK-3α involved in cancer cell proliferation and survival? What are the other cellular targets of GSK-3 that will lend proliferative and survival advantages to cancer cells? Future studies aimed at answering these questions will no doubt shed light on the multiple mechanisms by

Executive summary

Glycogen synthase kinase-3 & human diseases

- A large body of evidence supports the suggestion that pharmacological inhibitors of glycogen synthase kinase (GSK)-3 could be useful in the treatment of Alzheimer's disease and other neurodegenerative diseases, as well as in regulating inflammation and cancer.

GSK-3 & cancer: a paradigm with a controversial history

- Because GSK-3 inhibition are expected to mimic wingless-type (Wnt) signaling and stabilize oncogenic proteins, anti-GSK-3 therapy raises physician concerns that inhibition of this enzyme could presumably lead to tumorigenesis. However, Wnt signaling, cyclin D1 levels or β-catenin accumulation are not perturbed in GSK-3β-deficient mice.
- GSK-3 is not mutated in human cancer.
- The activities of GSK-3β, Akt and β-catenin is independent in colon cancer cell lines and human colorectal carcinomas.
- The risk of cancer development in psychiatric patients treated with lithium carbonate, a GSK-3 inhibitor used for bipolar disorder treatment, is significantly lower than in the general population.

Inhibition of GSK-3β suppresses cancer cell proliferation & survival by abrogating nuclear facotr κB nuclear activity

- Similar to the disruption of the nuclear factor *(NF)κB p65* or *inhibitor of κ B (IκB) kinase (IKK)β* genes, ablation of the murine *GSK-3β* gene resulted in embryonic lethality due to hepatocyte apoptosis and massive liver degeneration. These findings suggest *GSK-3β* involvement in the mechanism of NFκB activation.
- NFκB is constitutively active in most human hematopoietic and solid malignant tumors. NFκB activation is a part of the cancer cell's autodefense mechanism and thus may mediate tumor chemoresistance and radioresistance. Moreover, most chemotherapeutic agents and radiation that induce apoptosis also activate NFκB. Suppression of NFκB in tumors inhibits metastasis, causes cell-cycle arrest and leads to apoptosis.
- NFκB-mediated cancer cell survival and proliferation can be suppressed by inhibition of GSK-3β *in vitro* and *in vivo*.
- GSK-3β is required for NFκB nuclear activity independent of IκB degradation and NFκB nuclear translocation. A subset of anti-apoptotic (*XIAP*, *Bcl-2*) and proliferation (*cyclin D1*) genes required functional GSK-3β for NFκB-mediated transcriptional activation in human cancer. The exact mechanism by which GSK-3β impacts NFκB activity is unknown.
- Increased levels of β-catenin can antagonize NFκB activity. GSK-3α/β may contribute to constitutive NFκB transcriptional activity by targeting β-catenin for degradation, thereby preventing the formation of a β-catenin–NFκB interaction.

GSK-3 & carbohydrate metabolism in cancer

- Most tumors rely on anaerobic glycolysis even in the presence of abundant oxygen, known as a Warburg effect phenomenon. Malignant cells have the ability to compete for glucose up to 50-times more intensely than the surrounding normal tissue.
- GSK-3 inhibits the synthesis of glycogen, the storage form of carbohydrate in the human body. Expression of glycogen is significantly decreased in poorly differentiated tumors that show the highest proliferation rate.
- Inhibition of GSK-3 may affect glucose metabolism in cancer cells by upregulation of glycogen synthesis, leading to alterations in catabolic (anaerobic glycolysis) and anabolic (nucleotides synthesis) processes in the cancer cell, which may result in decreased cancer cell proliferation and survival.

Expression & localization of GSK-3β in cancer cells

- GSK-3β protein overexpression or nuclear localization was found in human ovarian, colon and pancreatic carcinomas.

Inhibition of GSK-3 by cyclin-dependent kinase inhibitors: side or major anticancer effect?

- GSK-3 inhibition is a side effect of numerous drugs used for cancer treatment.
- Most reported cyclin-dependent kinase (CDK) inhibitors are powerful GSK-3 inhibitors. The mechanism by which CDK inhibitors induced apoptosis in cancer cells is unknown. It is likely that CDK inhibitors induce apoptosis through inhibition of GSK-3, resulting in decreased NFκB transcriptional activity in cancer cells.
- Previously published studies on the cellular effects of CDK inhibitors need to be re-evaluated in terms of GSK-3 inhibition and its effect on cancer cell proliferation and survival.

Future perspective

- Inhibition of GSK-3 suppresses tumor growth, suggesting that the GSK-3β inhibitor may be used as a monotherapy for cancers with constitutively active NFκB.
- Given that NFκB activation mediates tumor chemoresistance and radioresistance, further studies are needed to investigate whether GSK-3 inhibition is useful in combination with anticancer drugs and radiotherapy.
- Further investigations are required to determine the mechanism of GSK-3β nuclear accumulation, its role in NFκB nuclear activity in cancer cells and whether GSK-3β regulates additional molecular pathways that contribute to human cancer.

which GSK-3 contributes to the pathogenesis of human cancer. Although the inhibition of GSK-3 suppresses tumor growth, suggesting that GSK-3 inhibitors may be used as a monotherapy for cancers with constitutively active NFκB, GSK-3 inhibition may be useful in combination with other anticancer drugs and radiotherapy, since it may help to sensitize the cancer cells to the effects of these agents by blocking NFκB activity. In view of its effect in human cancer, therapeutic inhibition of GSK-3 is an attractive goal, and with more than 50 inhibitors of GSK-3 already identified, we believe that clinical trials targeting GSK-3 in human cancer will begin in the near future.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Plyte SE, Hughes K, Nikolakaki E, Pulverer BJ, Woodgett JR: Glycogen synthase kinase-3: functions in oncogenesis and development. *Biochim. Biophys. Acta.* 1114(2–3), 147–162 (1992).
2. Doble BW, Woodgett JR: GSK-3: tricks of the trade for a multi-tasking kinase. *J. Cell Sci.* 116(Pt 7), 1175–1186 (2003).
   - •• Review article outlining the current understanding of the role of glycogen synthase kinase (GSK)-3 in numerous signaling pathways and human pathology.
3. Brion JP, Anderton BH, Authelet M *et al.*: Neurofibrillary tangles and tau phosphorylation. *Biochem. Soc. Symp.* 67, 81–88 (2001).
4. Kaidanovich O, Eldar-Finkelman H: The role of glycogen synthase kinase-3 in insulin resistance and type 2 diabetes. *Exp. Opin. Ther. Targets* 6(5), 555–561 (2002)
5. Parker PJ, Caudwell FB, Cohen P: Glycogen synthase from rabbit skeletal muscle; effect of insulin on the state of phosphorylation of the seven phosphoserine residues *in vivo*. *Eur. J. Biochem.* 130(1), 227–234 (1983).
6. Martin M, Rehani K, Jope RS, Michalek SM: Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3. *Nature Immunol.* 6(8), 777–784 (2005).
7. Dugo L, Collin M, Allen DA *et al.*: GSK-3β inhibitors attenuate the organ injury/dysfunction caused by endotoxemia in the rat. *Crit. Care Med.* 33(9), 1903–1912 (2005).
8. Polakis P: Wnt signaling and cancer. *Genes Dev.* 14, 1837–1851 (2000).
9. Hoeflich KP, Luo J, Rubie EA, Tsao MS, Jin O, Woodgett JR: Requirement for glycogen synthase kinase-3β in cell survival and NF-κB activation. *Nature* 406, 86–90 (2000).
   - •• Initial report demonstrating that ablation of GSK-3β results in embryonic lethality due to tumor necrosis factor (TNF)-α-dependent hepatocyte apoptosis, a phenotype similar to that of mice lacking the p65 nuclear factor (NF)κB subunit or Iκkinaseβ. This study pointed to an unexpected role for GSK-3β in NFκB nuclear activity.
10. Ding VW, Chen RH, McCormick F: Differential regulation of glycogen synthase kinase 3beta by insulin and Wnt signaling. *J. Biol. Chem.* 275, 32475–32481 (2000).
11. Patel S, Doble B, Woodgett JR: Glycogen synthase kinase-3 in insulin and Wnt signalling: a double-edged sword? *Biochem. Soc. Trans.* 32(Pt 5), 803–808 (2004).
12. Altomare DA, Testa JR: Perturbations of the Akt signaling pathway in human cancer. *Oncogene* 24, 7455–7464 (2005).
13. Cheng JQ, Ruggeri B, Klein WM *et al.*: Amplification of AKT2 in human pancreatic cells and inhibition of AKT2 expression and tumorigenicity by antisense RNA. *Proc. Natl Acad. Sci. USA* 93(8), 3636–3641 (1996).
14. Ougolkov AV, Fernandez-Zapico ME, Savoy DN, Urrutia RA, Billadeau DD: Glycogen synthase kinase-3β participates in nuclear factor-κB-mediated gene transcription and cell survival in pancreatic cancer cells. *Cancer Res.* 65, 2076–2081 (2005).
   - •• Initial report showing that pharmacological inhibition of GSK-3 or genetic depletion of GSK-3β decreases NFκB-mediated pancreatic cancer cell proliferation and survival. This study suggests GSK-3β as a potential therapeutic target in the treatment of pancreatic cancer.
15. Shakoori A, Ougolkov A, Yu ZW *et al.*: Deregulated GSK3β activity in colorectal cancer: Its association with tumor cell survival and proliferation. *Biochem. Biophys. Res. Commun.* 334, 1365–1374 (2005).
   - • Suggests GSK-3β as a potential therapeutic target in colorectal cancer. Levels of GSK-3β and the two phosphorylated forms were analyzed by Western blotting in pairs of normal and tumor tissues of colorectal cancer patients (n = 20 cases). The level of GSK-3β in the tumors was higher than in their normal counterparts in 18 out of 20 cases. Inactive phospho-GSK-3β Ser9 was detected in high levels in non-neoplastic tissue in 17 out of 20 cases, whereas tumors in most cases contained only trace amounts or no detectable level of this form. Moreover, this study demonstrates that inhibition of GSK-3 or genetic depletion of GSK-3β by RNA interference induces apoptosis and attenuates proliferation of colon cancer cells.
16. Ryves WJ, Harwood AJ: The interaction of glycogen synthase kinase-3 (GSK-3) with the cell cycle. *Prog. Cell Cycle Res.* 5, 489–495 (2003).
17. Gould TD, Gray NA, Manji HK: Effects of a glycogen synthase kinase-3 inhibitor, lithium, in adenomatous polyposis coli mutant mice. *Pharmacol. Res.* 48(1), 49–53 (2003).
18. Cohen Y, Chetrit A, Cohen Y, Sirota P, Modan B: Cancer morbidity in psychiatric patients: influence of lithium carbonate treatment. *Med. Oncol.* 15(1), 32–36 (1998).
19. Erdal E, Ozturk N, Cagatay T, Eksioglu-Demiralp E, Ozturk M: Lithium-mediated downregulation of PKB/Akt and cyclin E with growth inhibition in hepatocellular carcinoma cells. *Int. J. Cancer* 115(6), 903–910 (2005).
20. Feinman R, Koury J, Thames M, Barlogie B, Epstein J, Siegel DS: Role of NF-κB in the rescue of multiple myeloma cells from glucocorticoid-induced apoptosis by bcl-2. *Blood* 93(9), 3044–3052 (1999).
21. Griffin JD: Leukemia stem cells and constitutive activation of NF-κB. *Blood* 98(8), 2291 (2001).
22. Kordes U, Krappmann D, Heissmeyer V, Ludwig WD, Scheidereit C: Transcription factor NF-κB is constitutively activated in acute lymphoblastic leukemia cells. *Leukemia* 14(3), 399–402 (2000).
23. Baron F, Turhan AG, Giron-Michel J *et al.*: Leukemic target susceptibility to natural killer cytotoxicity: relationship with BCR-ABL expression. *Blood* 99(6), 2107–2113 (2002).
24. Palayoor ST, Youmell MY, Calderwood SK, Coleman CN, Price BD: Constitutive activation of IκB kinase α and NF-κB in prostate cancer cells is inhibited by ibuprofen. *Oncogene* 18(51), 7389–7394 (1999).

25. Nakshatri H, Bhat-Nakshatri P, Martin DA, Goulet RJ Jr, Sledge GW Jr: Constitutive activation of NF-κB during progression of breast cancer to hormone-independent growth. *Mol. Cell Biol.* 17(7), 3629–3639 (1997).
26. Wang W, Abbruzzese JL, Evans DB, Larry L, Cleary KR, Chiao PJ: The nuclear factor-κB RelA transcription factor is constitutively activated in human pancreatic adenocarcinoma cells. *Clin. Cancer Res.* 5, 119–127 (1999).
27. Beg AA, Baltimore D: An essential role for NF-κB in preventing TNF-α-induced cell death. *Science* 274(5288), 782–784 (1996).
28. Wang CY, Cusack JC Jr, Liu R, Baldwin AS Jr: Control of inducible chemoresistance: enhanced anti-tumor therapy through increased apoptosis by inhibition of NF-κB. *Nature Med.* 5(4), 412–417 (1999).
29. Pahl HL: Activators and target genes of Rel/NF-κB transcription factors. *Oncogene* 18, 6853–6866 (1999).
30. Andela VB, Schwarz EM, Puzas JE, O'Keefe RJ, Rosier RN: Tumor metastasis and the reciprocal regulation of prometastatic and antimetastatic factors by nuclear factor κB. *Cancer Res.* 60, 6557–6562 (2000).
31. Aggarwal BB: Nuclear factor-*k*B: the enemy within. *Cancer Cell* 6(3), 203–208 (2004).
•• Review article, outlining the role of NFκB activation in human cancer suggesting inhibition of NFκB as an effective method in the prevention and treatment of cancer.
32. Beg AA, Sha WC, Bronson RT, Ghosh S, Baltimore D: Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B. *Nature* 376(6536), 167–170 (1995).
33. Li Q, Van Antwerp D, Mercurio F, Lee KF, Verma IM:Severe liver degeneration in mice lacking the *IκB kinase 2* gene. *Science* 284(5412), 321–325 (1999).
34. Beyaert R, Vanhaesebroeck B, Suffys P, Van Roy F, Fiers W: Lithium chloride potentiates tumor necrosis factor-mediated cytotoxicity *in vitro* and *in vivo*. *Proc. Natl Acad. Sci. USA* 86(23), 9494–9498 (1989).
35. Mazor M, Kawano Y, Zhu H, Waxman J, Kypta RM: Inhibition of glycogen synthase kinase-3 represses androgen receptor activity and prostate cancer cell growth. *Oncogene* 23(47), 7882–7892 (2004).
• Shows that inhibition of GSK-3 leads to a reduction both in androgen receptor (AR) protein levels and the growth of AR-positive prostate cancer cell lines, suggesting a novel therapeutic application for GSK-3 inhibitors in prostate cancer.
36. Liao X, Zhang L, Thrasher JB, Du J, Li B: Glycogen synthase kinase-3beta suppression eliminates tumor necrosis factor-related apoptosis-inducing ligand resistance in prostate cancer. *Mol. Cancer Ther.* 2(11), 1215–1222 (2003).
37. Ghosh JC, Altieri DC: Activation of p53-dependent apoptosis by acute ablation of glycogen synthase kinase-3β in colorectal cancer cells. *Clin. Cancer Res.* 11(12), 4580–4588 (2005).
38. Collett GP, Campbell FC: Curcumin induces c-jun N-terminal kinase-dependent apoptosis in HCT116 human colon cancer cells. *Carcinogenesis* 25(11), 2183–2189 (2004).
39. Thisse C, Perrin-Schmitt F, Stoetzel C, Thisse B: Sequence-specific transactivation of the *Drosophila twist* gene by the dorsal gene product. *Cell* 65(7), 1191–1201 (1991).
40. Tergaonkar V, Pando M, Vafa O, Wahl G, Verma I: p53 stabilization is decreased upon NFκB activation: a role for NFκB in acquisition of resistance to chemotherapy. *Cancer Cell* 1(5), 493–503 (2002).
41. Deng J, Xia W, Miller SA, Wen Y, Wang HY, Hung MC: Crossregulation of NF-kappaB by the APC/GSK-3β/β-catenin pathway. *Mol. Carcinog.* 39(3), 139–146 (2004).
42. Deng J, Miller SA, Wang HY *et al.*: β-catenin interacts with and inhibits NF-κB in human colon and breast cancer. *Cancer Cell* 2(4), 323–334 (2002).
43. Steinbrecher KA, Wilson W 3rd, Cogswell PC, Baldwin AS: Glycogen synthase kinase 3[β] functions to specify gene-specific, NF-[κ]B-dependent transcription. *Mol. Cell Biol.* 25(19), 8444–8455 (2005).
•• Identifies a subset of NFκB-regulated genes that require GSK-3β for efficient expression and demonstrates that GSK-3β has a profound effect on NFκB transcription in a gene-specific manner through a mechanism involving control of promoter-specific recruitment of NFκB.
44. Schwabe RF, Brenner DA: Role of glycogen synthase kinase-3 in TNF-α-induced NF-κB activation and apoptosis in hepatocytes. *Am. J. Physiol. Gastrointest. Liver Physiol.* 283(1), G204–G211 (2002).
45. Buss H, Dorrie A, Schmitz ML *et al.*: Phosphorylation of serine 468 by GSK-3β negatively regulates basal p65 NF-κB activity. *J. Biol. Chem.* 279(48). 49571–49574 (2004).
46. Bachelder RE, Yoon SO, Franci C, de Herreros AG, Mercurio AM: Glycogen synthase kinase-3 is an endogenous inhibitor of Snail transcription: implications for the epithelial-mesenchymal transition. *J. Cell. Biol.* 168(1), 29–33 (2005).
47. Warburg O: *The metabolism of tumors*. Constable Press, London (1930).
48. Warburg O: On the origin of cancer cells. *Science* 123, 309–314 (1956).
49. Garber K: Energy boost: The Warburg effect returns in a new theory of cancer. *J. Natl Cancer Inst.* 96, 1805–1806 (2004).
50. Gatenby RA, Gawlinski ET: The glycolytic phenotype in carcinogenesis and tumor invasion: insights through mathematical models. *Cancer Res.* 63, 3847–3854 (2003).
•• Describes the benefits of anaerobic glycolysis for cancer cells in terms of tumor invasion, increased proliferation and vascularization in a tumor–host interaction model.
51. Namiot Z, Stasiewicz J, Szalaj W, Skwarski L, Jodczyk J, Gorski J: Gastric cancer with special references to WHO and Lauren's classifications: glycogen and triacylglycerol concentrations in the tumor. *Neoplasma* 36, 363–368 (1989).
52. Takahashi S, Satomi A, Yano K *et al.*: Estimation of glycogen levels in human colorectal cancer tissue: relationship with cell cycle and tumor outgrowth. *J. Gastroenterol.* 34, 474–480 (1999).
53. Rask K, Nilsson A, Brannstrom M *et al.*: Wnt-signalling pathway in ovarian epithelial tumours: increased expression of β-catenin and GSK3β. *Br. J. Cancer* 89(7), 1298–1304 (2003).
• Expression of GSK-3β was analyzed by Western blotting and statistical analysis, revealing significant increase in GSK-3β ($p < 0.001$) expression in the group of ovarian adenocarcinomas compared with the group of normal ovaries and benign adenomas/borderline tumors.
54. Gotoh J, Obata M, Yoshie M, Kasai S, Ogawa K: Cyclin D1 overexpression correlates with β-catenin activation, but not with H-ras mutations, and phosphorylation of Akt, GSK3 β and ERK1/2 in mouse hepatic carcinogenesis. *Carcinogenesis* 24(3), 435–442 (2003).
55. Beals CR, Sheridan CM, Turck CW, Gardner P, Crabtree GR: Nuclear export of NF-ATc enhanced by glycogen synthase kinase-3. *Science* 275, 1930–1934 (1997).
56. Wei W, Jin J, Schlisio S, Harper JW, Kaelin WG Jr:The v-Jun point mutation allows c-Jun to escape GSK3-dependent recognition and destruction by the Fbw7 ubiquitin ligase. *Cancer Cell* 8(1), 25–33 (2005).

57. De Ketelaere A, Vermeulen L, Vialard J *et al.*: Involvement of GSK-3β in TWEAK-mediated NF-κB activation. *FEBS Lett.* 566(1–3), 60–64 (2004).

58. Senderowicz AM: Novel small molecule cyclin-dependent kinases modulators in human clinical trials. *Cancer Biol. Ther.* 2(4 Suppl. 1), S84–S95 (2003).

59. Leclerc S, Garnier M, Hoessel R *et al.*: Indirubins inhibit glycogen synthase kinase-3β and CDK5/p25, two protein kinases involved in abnormal tau phosphorylation in Alzheimer's disease. A property common to most cyclin-dependent kinase inhibitors? *J. Biol. Chem.* 276(1), 251–260 (2001).

•• Describes most cyclin-dependent kinase inhibitors as powerful GSK-3 inhibitors and raises the question of dual-specificity effect of these drugs in the treatment of human cancer.

60. Kim DM, Koo SY, Jeon K *et al.*: Rapid induction of apoptosis by combination of flavopiridol and tumor necrosis factor (TNF)-α or TNF-related apoptosis-inducing ligand in human cancer cell lines. *Cancer Res.* 63(3), 621–626 (2003).

61. Kitada S, Zapata JM, Andreeff M, Reed JC: Protein kinase inhibitors flavopiridol and 7-hydroxy-staurosporine down-regulate antiapoptosis proteins in B-cell chronic lymphocytic leukemia. *Blood* 96(2), 393–397 (2000).

62. Wittmann S, Bali P, Donapaty S *et al.*: Flavopiridol down-regulates antiapoptotic proteins and sensitizes human breast cancer cells to epothilone B-induced apoptosis. *Cancer Res.* 63(1), 93–99 (2003).

63. Salgia R, Skarin AT: Molecular abnormalities in lung cancer. *J. Clin. Oncol.* 16(3), 1207–1217 (1998).

64. Joseph B, Marchetti P, Formstecher P, Kroemer G, Lewensohn R, Zhivotovsky B: Mitochondrial dysfunction is an essential step for killing of non-small cell lung carcinomas resistant to conventional treatment. *Oncogene* 21(1), 65–77 (2002).

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