Primers on Molecular Pathways

The Glycogen Synthase Kinase-3 β

Daniel D. Billadeau

Department of Immunology and Oncology Research, Mayo Clinic College of Medicine, Rochester, Minn., USA

Key Words

Glycogen synthase kinase-3 • Glycogen synthase • β -Catenin • NF κ B • Pancreatic adenocarcinoma • Heterochromatin

Abstract

Despite tremendous scientific effort, conventional treatment approaches have had little impact on the course of pancreatic ductal adenocarcinoma. Therefore, urgency is needed to understand the molecular mechanisms underlying the development of pancreatic cancer with the hope that this will lead to preventative and treatment strategies to improve the outcome of the disease. Numerous factors contribute to progression of this disease, including constitutively active NF κ B, which has been shown to positively influence cancer cell survival, proliferation, invasion, metastasis and chemoresistance. Recently, the cytoplasmic serine/threonine protein kinase glycogen synthase kinase-3 β (GSK-3 β) was found to regulate NF κ B activation and the proliferation and survival of pancreatic cancer cells. Moreover, recent studies in other human malignancies have implicated GSK-3 β as a regulator of cancer cell proliferation, survival and chemoresistance through distinct mechanisms. Thus, GSK-3 β has emerged as a viable therapeutic target in the treatment of several human neoplasms.

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Pancreatic ductular adenocarcinoma is the most common pancreatic tumor, accounting for more than 90% of all pancreatic cancers and ranks fourth as a cause of death by cancer in the USA [1]. Pancreatic cancer has one of the poorest prognoses among human neoplasms, with an overall 5-year survival rate of 3–5% [1]. This is due to the highly aggressive and rapidly metastatic nature of the disease and the fact that current diagnostic tools do not detect early stages of the disease, as indicated by the reality that many patients have metastases at the time of diagnosis. Pancreatic cancer is also essentially treatment-resistant, in that conventional chemotherapy makes little impact on the course of disease. Therefore, efforts to understand the molecular mechanisms underlying the development of pancreatic cancer may lead to prevention and treatment strategies that improve the prognosis of the disease.

Although the underlying etiology and pathophysiology of pancreatic ductal cancer are poorly understood, there is increasing evidence that signaling and transcriptional pathways that control cell proliferation, differentiation, and apoptosis are deregulated in pancreatic cancer [2]. Together, changes in these molecular pathways lead to abnormalities in growth factor-mediated signaling cascades, cell cycle control, cellular migration, and transcriptional regulation. For example gain-of-function mutations in the *K-ras* proto-oncogene, a key regulator of

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1424–3903/07/0076–0398\$23.50/0

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Daniel D. Billadeau, PhD
Department of Immunology and Division of Oncology Research
200 First Street SW
Rochester, MN 55905 (USA)
Tel. +1 507 266 4334, Fax +1 507 266 5146, E-Mail billadeau.daniel@mayo.edu

cell proliferation, are among the most common genetic abnormalities associated with pancreatic cancer [3]. In addition, antiproliferative pathways (e.g. TGF- β and DPC4) and those that regulate programmed cell death (e.g. p53) are frequently altered in pancreatic cancer [4]. Along with deregulation of these pathways, constitutive activation of NF κ B is a frequent occurrence in pancreatic cancer [5], leading to transcriptional activation of genes involved in cell proliferation, survival, angiogenesis and chemoresistance [6–8]. These protumorigenic effects of NF κ B have made it an attractive chemotherapeutic target in many human malignancies, including pancreatic cancer.

NF κ B, p65/p50 heterodimers are generally found in a complex with I κ B α in the cytosol of resting cells, where I κ B α sequesters p65/p50 by masking a nuclear localization signal sequence found on p65 [6, 9]. Following receptor stimulation (e.g. TNFR), the IKK complex (IKK α , β , γ /Nemo) is activated, resulting in the phosphorylation of I κ B α , leading to its ubiquitylation and degradation. This exposes the nuclear localization signal sequence on p65, resulting in the nuclear translocation of p65/p50, and, following chromatin remodeling, transcriptional activation of NF κ B target gene promoters [6, 9]. It is currently unclear which molecular pathways are deregulated in pancreatic cancer leading to constitutive activation of NF κ B, but they may involve not only constitutive ligand/receptor signaling leading to IKK activation, but also the activation of other pathways, including those involved in chromatin remodeling, which cooperate with NF κ B to drive expression of its target genes.

Glycogen synthase kinase-3 (GSK-3) is a ubiquitously expressed serine/threonine kinase originally identified as a regulator of glycogen synthesis [10]. There are two highly homologous mammalian isoforms encoded by different genes (GSK-3 α and GSK-3 β), which share substrate specificity *in vitro* and are involved in regulating cell fate determination and differentiation in a variety of organisms [10]. GSK-3 β both positively and negatively regulates the activity of a broad range of substrates by phosphorylation [11]. Classically, GSK-3 proteins are best known for their role in the regulation of glycogen synthase and β -catenin [11]. GSK-3 kinases control glucose metabolism through their phosphorylation and inactivation of glycogen synthase. This inhibitory activity is inactivated by insulin signaling through phosphorylation and inactivation of GSK-3 by the kinase Akt [12]. GSK-3 kinases are also a key component of the β -catenin destruction complex, where they regulate the accumulation and nuclear translocation of the oncogene β -catenin

through specific phosphorylation of β -catenin, leading to its ubiquitylation and degradation [13, 14]. Signaling through the Wnt family receptors leads to the dissolution of the β -catenin destruction complex, and ultimately, β -catenin accumulation, nuclear translocation and transcriptional activation of genes involved in proliferation, differentiation and survival [13]. Thus, GSK-3 kinases serve a critical role in cellular homeostasis and glucose metabolism, as well as preventing tumorigenesis through their regulatory effects on β -catenin.

Surprisingly, although several members of the β -catenin destruction complex, including the adenomatous polyposis coli tumor suppressor, axin and β -catenin have been found mutated in colon cancer, inactivating mutations in either of the GSK-3 kinase genes have not been observed [15]. In fact, in contrast to what would be predicted, overexpression of active GSK-3 β has been found in human colon cancer tissues, which harbor constitutively active Akt, as well as β -catenin nuclear accumulation [16], suggesting that GSK-3 β may participate in colon cancer tumorigenesis, independent of its effects on β -catenin. Consistent with this notion, depletion of GSK-3 β by RNA interference, or pharmacological inhibition of GSK-3 kinase activity results in decreased survival and proliferation of colon cancer cells [16]. In addition to participating in colon cancer tumorigenesis, we have recently demonstrated a critical role for GSK-3 β in the proliferation and survival of pancreatic cancer cells [17, 18] and other investigators have shown roles for GSK-3 β in the regulation of proliferation and survival of hepatocellular [19], ovarian [20], prostate [21] and AML [22] cancer cells. However, how GSK-3 β contributes to the tumorigenic phenotype in these various human cancers remains ill defined, but may involve its positive regulatory effects on gene transcription.

Disruption of the murine *gsk-3 β* gene results in embryonic lethality, and mouse embryonic fibroblasts (MEFs) derived from these animals are more sensitive to TNF- α -induced apoptosis [23]. This sensitivity was found to be due to an inability to activate NF κ B-mediated gene transcription, thus implicating a role for GSK-3 β , but not GSK-3 α , in the regulation of NF κ B transcriptional activity [23]. Although GSK-3 β does not harbor an identifiable nuclear localization signal sequence, it was found to shuttle in and out of the nucleus [24], and can do so in the human 293 kidney epithelial cell line and MEFs in response to TNF- α stimulation (unpubl. observation). Additionally, we have found that GSK-3 β accumulates in the nucleus of pancreatic cancer cells [18] and CLL B cells [25], two tumor types in which NF κ B is known to accu-

Fig. 1. Possible mechanisms by which GSK-3 β regulates NF κ B-mediated gene transcription. GSK-3 β accumulates in the nucleus in pancreatic cancer cells and in response to TNF- α stimulation. (1) GSK-3 β has been shown to phosphorylate and stabilize p105, thus providing a pool from which p50 can be generated following degradation. (2) Additionally, GSK-3 β has been found to phosphorylate p65, which might affect its ability to interact with co-activators such as the histone acetyl transferase (HAT) p300, thereby preventing DNA binding and transcriptional activation.

(3) GSK-3 β might affect the activity of HDACs or HMTs, thereby maintaining an open (acetylated) euchromatin configuration at NF κ B target gene promoters through the recruitment of HATs, which will facilitate p65/p50 binding and transcriptional activation. (4) Inhibition of GSK-3 β might lead to increased activity of HDACs and HMTs, resulting in deacetylation and the subsequent hypermethylation of the histone tails and the formation of heterochromatin.

mulate in the nucleus and transactivate target genes involved in cell survival. It is therefore tempting to speculate that this nuclear accumulation of GSK-3 β is required for optimal NF κ B transcriptional activity in normal cell signaling, as well as in cancer, yet the molecular mechanisms by which receptor signaling leading to NF κ B activation and the nuclear translocation of GSK-3 β are tethered remain to be determined.

The exact mechanism by which nuclear GSK-3 β could alter NF κ B-mediated gene transcription is unclear, but

might involve regulation of transcriptional binding partners through phosphorylation (fig. 1). In fact, while GSK-3 β is known to positively influence NF κ B transcriptional activity, GSK-3 β has been shown to directly phosphorylate p65 on serine 468 within the transactivation domain, resulting in decreased NF κ B transcriptional activation [26]. However, a more recent study indicates that serine 468 is phosphorylated by IKK β , not GSK-3 β [27]. Additionally, GSK-3 β has also been shown to phosphorylate p65 within a region encompassing the transactivation

domain [28]; however, the exact site of phosphorylation was not mapped. Lastly, it has been reported that GSK-3 β phosphorylates p105 and is involved in the stabilization of p105, the precursor protein to p50 (a component of active NF κ B; p65/p50) in resting cells, and the degradation of p105 to p50 in response to TNF- α stimulation [29]. In this case, GSK-3 β would be involved in regulating the levels of active p65/p50 complexes, which could subsequently transactivate NF κ B target genes. However, TNF- α -stimulated expression of the NF κ B target gene I κ B α requires the binding of both p65 and p50, and expression of this target gene is unaffected in the absence of GSK-3 β protein [30, 31].

Recently, it was shown that while p65/p50 accumulates in the nucleus of GSK-3 β -deficient MEFs following TNF- α stimulation, p65 was not able to interact with certain target gene promoters, as determined by chromatin immunoprecipitation [31]. This suggests that either p65 does not have access to its target promoters, or, once bound to the promoter, it is rapidly removed. Indeed, remodeling of the chromatin at NF κ B target gene promoters occurs following TNF- α stimulation and is a requisite event leading to binding of NF κ B to the promoter and transcriptional activation [29]. Thus, it remains possible that GSK-3 β localizes to the nucleus in response to TNF- α signaling in order to modulate proteins involved in chromatin remodeling leading to the formation of euchromatin at NF κ B target gene promoters. This is interesting in light of a recent observation identifying that histone deacetylase 1 (HDAC1), but not HDAC2, HDAC3 or HDAC8, is specifically degraded following TNF- α stimulation in an IKK-dependent manner [32]. It is possible that GSK-3 β works along with the IKK complex to target HDAC1 for degradation in response to TNF- α stimulation, thereby permitting acetylation of histones at NF κ B target promoters leading to the formation of euchromatin and, ultimately, p65/p50 binding and transcriptional activation.

In pancreatic cancer cells, it is possible that constitutive signaling to NF κ B and the nuclear accumulation of GSK-3 β result in the maintenance of euchromatin at NF κ B target gene promoters, thereby allowing high-level transcriptional activation. In fact, we have found by chromatin immunoprecipitation that histone H3 at NF κ B target gene promoters is hyperacetylated on K9, and K14 (unpubl. observation). However, upon inhibition of GSK-3, K9 and K14 lose their acetylation and become methylated (unpubl. observation), suggesting that GSK-3 might be involved in maintaining the euchromatic state of NF κ B target gene promoters in pancreatic cancer cells.

We have also found that GSK-3 inhibition in CLL B cells results in the hypermethylation of histone H3 (K9 and K27) within the XIAP and Bcl-2 promoters and loss of p65/p50 binding and transcriptional activation [25]. Taken together, these data offer the attractive hypothesis that GSK-3 β regulates NF κ B transcriptional activity by preventing the activity of HDACs or histone methyltransferases (HMTs) at target gene promoters (fig. 1). However, it is not yet clear if the epigenetic changes occurring within these promoters in response to GSK-3 inhibition are due to the loss of p65/p50 binding or are the consequence of that loss. In fact, it remains possible that inhibition of GSK-3 affects the formation of stable p65/p50 complexes on DNA by preventing the association with p300 or other cofactors, leading to its loss from the promoter and then the formation of heterochromatin.

GSK-3 β has been primarily investigated regarding its ability to phosphorylate and inactivate or degrade specific target substrates, many of them associated with cellular transformation (β -catenin, cyclin D1, c-myc, NFAT) [11]. However, pharmacological inactivation of GSK-3 as well as RNAi-mediated gene silencing of GSK-3 β have demonstrated a critical role for this kinase in the proliferation and survival in several human malignancies [15]. Moreover, recent studies indicate that GSK-3 β is overexpressed in many human malignancies [16, 18], where it can be found accumulated in the nucleus [18, 25]. It is quite possible that this nuclear accumulation is important for its effects on the transcriptional activity of NF κ B. In addition, it is likely that GSK-3 β positively influences the transcriptional activity of other transcription factors either directly, or through its effects on HDACs and/or HMTs. While more work needs to be done to understand the mechanism by which GSK-3 β contributes to pancreatic tumorigenesis and NF κ B transcriptional activity, it is clear that GSK-3 β has emerged as a therapeutic target for the treatment of this devastating disease.

Acknowledgements

This work was supported in part by the Mayo Foundation, and a Specialized Program of Research Excellence (SPORE) grant in pancreatic cancer (P50 CA102701) to D.D.B.

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