KRAS-activating mutations have long been implicated in different human cancers, particularly a form of pancreatic cancer called pancreatic ductal adenocarcinoma (PDAC). However, attempts to target KRAS therapeutically have proven to be a challenge. Therefore, understanding downstream effectors may illuminate new targets and strategies. Previous studies have shown that activated KRAS turns on many downstream signaling pathways, including phosphoinositide 3-kinase (PI3K)/AKT, mitogen-activated protein kinase (MAPK), and NF-κB (1). Here, Bang and colleagues (2) report a new mediator of oncogenic KRAS in PDAC, GSK-3β, that activates not only canonical but also noncanonical NF-κB signaling pathways, thereby potentially revealing a new therapeutic target for PDAC.

It has previously been shown that KRAS activates NF-κB, which is crucial for cell survival and tumor transformation, along with concomitant p53 loss (3). Others have attempted to understand the intermediate players between KRAS-activating mutations and NF-κB signaling. It has also been shown that the signaling adaptor p62 is required for both RAS-induced transformation and NF-κB activation in a lung tumor model (1). The induction of p62 occurred through an ERK- and AKT-dependent pathway, which then activated TRAF6 E3-ubiquitin ligase activity as well as NF-κB via phosphorylation of the upstream kinase IκB kinase (IKKα/β). TBK1, a serine-threonine kinase that can activate NF-κB, was also found to be a synthetically lethal partner with mutant KRAS (4). Bang and colleagues (2) now introduce GSK-3β into the fray.

The study of glycogen synthase kinase-3 (GSK-3) spans over 30 years since its discovery as one of several protein kinases that phosphorylate glycogen synthase. GSK-3 is now known to be involved in myriad biologic functions, including glycogen metabolism, Wnt and Hedgehog signal transduction, protein synthesis, mitosis, and apoptosis (5). There are 2 isoforms of GSK-3, α and β; they have masses of 51 and 47 kDa, respectively, and are encoded by different genes. GSK-3α contains a glycine-rich region at the N-terminus that GSK-3β does not. The 2 isoforms share 98% identity within their kinase domains; however, they share only 36% identity in the 76 C-terminal residues. GSK-3β also has a minor splice variant, GSK-3β2, which contains a 13-residue insert within the kinase domain. Most of the literature on GSK-3 focuses on the GSK-3β isoform, rather than GSK-3α. Gsk3b-null mice are embryonic lethal due to liver degeneration caused by TNF-α toxicity, whereas Gsk3a-null mice are viable but display enhanced glucose and insulin sensitivity, as well as reduced fat mass (6). These findings clearly highlight different physiologic roles that these proteins play. GSK-3β has been reported to mediate NF-κB activation and gene transcription to promote pancreatic cancer, osteosarcoma, oral cancer, certain leukemias, and gliomas, as well as other cancers (7, 8). The phenotype of Gsk3b-null mice is remarkably similar to that of p65-deficient and IKKβ-deficient mice, and fibroblasts from these mice also showed reduced NF-κB function, further establishing the genetic link between GSK-3β and NF-κB signaling. However, the role of GSK-3β in pro-cancer effects is thus far limited to the context of acute myeloid leukemia (9), and no clear link to NF-κB signaling has been revealed. By using siRNA specific to each GSK-3 isoform, Bang and colleagues (2) found that growth of pancreatic cancer cell lines harboring a KRAS mutation (PanC-1 and MiaPaCa-2) was reduced by inhibition of GSK-3α, whereas inhibition of GSK-3β had much less effect. Thus, the authors expanded the limited knowledge of GSK-3β by implicating its striking pro-tumorigenic role through NF-κB signaling in pancreatic cancer cells harboring oncogenic KRAS mutation (Fig. 1).

How does GSK-3α activate NF-κB and promote growth of KRAS-mutant pancreatic cancer cells? Knockdown of GSK-3α, but not GSK-3β, or treatment with the GSK-3 inhibitor AR-A014418, showed a decrease in TGF-β–activated kinase 1 (TAK1) levels, hinting at a novel mechanism at play. TAK1 initiates downstream NF-κB and MAPK signaling in association with its TAK1-binding partners (TAB1–4) in response to an array of extracellular signals and intracellular stress conditions. TAK1 leads to canonical activation of NF-κB by

**Summary:** Bang and colleagues report a novel role for GSK-3α, rather than the well-studied GSK-3β, as the link between oncogenic KRAS and the canonical and noncanonical activation pathways of NF-κB in pancreatic cancer. Although the mechanism through which it promotes noncanonical activation remains unclear, the authors show that GSK-3α binds and stabilizes TAK1-TAB complexes to constitutively activate canonical NF-κB signaling. Consequently, the inhibition of GSK-3α retards pancreatic cancer growth in vitro and in vivo, thereby revealing this relatively less-studied kinase as a potential therapeutic target for treatment of KRAS-positive pancreatic cancer.
Bang and colleagues (2) raises more questions than answers. The noncanonical activation pathway of NF-κB is depicted in yellow, and disease types are depicted in green. Bang and colleagues (2) introduced a new role for GSK-3α, which is implicated in many cancer and disease types. In contrast, the analysis of its relative, GSK-3β, has been rather limited. The molecular targets of GSK-3 are directly phosphorylating IKKβ and activating the IKK complex, which then phosphorylates the inhibitor of κB alpha (IκBα). IκBα is then degraded, and active NF-κB is liberated. TAK1 has previously been shown to be involved in the survival of colorectal cancers as well as pancreatic cancers (10, 11), but the mechanistic details have not been well elucidated. Consistent with GSK-3α being a downstream effector of KRAS, knockdown of KRAS in HPDE6KR+ cells reduced TAK1 protein levels. GSK-3α was found in a TAK1–TAB1 complex, and silencing of either GSK-3α or KRAS led to reduced TAK1–TAB1 interaction in HPDE6KR+ cells. As predicted, TAK1 inhibition by RNAi resulted in diminished phosphorylation of IκBα and p65/RelA. TAK1 inhibition with 5Z-7-oxozaenol led to decreased proliferation in pancreatic cancer cells via G1–M arrest with only a modest increase in apoptosis. These findings led the authors to propose that GSK-3α activates TAK1–TAB complexes by directly binding and stabilizing the complex to chronically stimulate canonical NF-κB signaling.

Surprisingly, the role of GSK-3α was not limited only to canonical NF-κB signaling. Bang and colleagues (2) found that the effect of TAK1 inhibition was consistently less than that of GSK-3 inhibition, suggesting the presence of additional downstream target(s) of GSK-3α. Therefore, they looked to the noncanonical activation pathway of NF-κB, which has also been implicated in pancreatic cancer. The noncanonical activation pathway involves NF-κB–inducing kinase activation of the IKK complex, which is composed of an IKKa dimer. The IKK complex phosphorylates p100, causing it to be processed to an active p52 subunit of NF-κB. Bang and colleagues (2) found that depletion of GSK-3α, but again not GSK-3β, led to a decrease in p100 to p52 processing in oncogenic KRAS-positive PDAC cells. Thus, the authors identified 2 new modes of GSK-3α action in pancreatic cancer cells, KRAS–GSK-3α–TAK1–IKKβ–canonical NF-κB and KRAS–GSK-3α–IKKα–noncanonical NF-κB pathways.

As with many studies that initiate a new road, the study by Bang and colleagues (2) raises more questions than answers. How does GSK-3α association with TAK1 promote stabilization and activation of the TAK1–TAB complex? Because the kinase activity of GSK-3α is important based on the chemical inhibitor analysis, GSK-3α presumably phosphorylates target protein(s). As there is no consensus substrate motif in TAK1 (S/T-XXX-S/T), the authors hypothesize that GSK-3α may phosphorylate TAB1 or TAB2, each of which harbors the consensus site. Most of the time, GSK-3 requires a primed substrate or a substrate that has already been phosphorylated at the fourth amino acid downstream of the target phosphorylation serine or threonine site; however, in some cases, such as β-catenin, this is not necessary. Other questions include: How does KRAS regulate the engagement of GSK-3α in the TAK1–TAB complex? How does GSK-3α mediate the noncanonical NF-κB signaling pathway? The authors provided evidence that this might occur within the nucleus. Why does GSK-3β fail to contribute in a similar way to KRAS-positive pancreatic cancer? Is there any critical amino acid motif present in GSK-3α but missing in GSK-3β which controls canonical and noncanonical NF-κB signaling? As mentioned above, there are significant differences in primary sequences between GSK-3α and β. Is the target of the TAK1/TAB complex limited only to the canonical NF-κB pathway, or is it phosphorylating other target substrates as well, such as extracellular signal-related kinase (ERK) or c-jun-NH2-kinase (JNK)? Finally, is the new role of GSK-3α revealed by Bang and colleagues (2) limited only to KRAS-positive pancreatic cancer or is this also relevant to other cancer types? While illuminating new therapeutic possibilities to target in pancreatic cancer, the study by Bang and colleagues (2) also opened up a new world of investigation into the less understood α isoform of GSK-3 and its role in different signaling pathways and perhaps other cancer types.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
Grant Support
This work was funded by NIH T32 GM008688, T32 CA009135, and R01 CA155192.

Published online June 7, 2013.

REFERENCES
A New Alpha in Line Between KRAS and NF-κB Activation?

Chorom Pak and Shigeki Miyamoto

*Cancer Discovery* 2013;3:613-615.

Updated version

Access the most recent version of this article at:
http://cancerdiscovery.aacrjournals.org/content/3/6/613

Cited articles

This article cites 11 articles, 4 of which you can access for free at:
http://cancerdiscovery.aacrjournals.org/content/3/6/613.full#ref-list-1

Citing articles

This article has been cited by 2 HighWire-hosted articles. Access the articles at:
http://cancerdiscovery.aacrjournals.org/content/3/6/613.full#related-urls

E-mail alerts

Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.