et al., 2006) to stick with a particular mode of movement irrespective of the external environment, but in general, the ability to switch between modes of movement gives cells the greatest chance to react to a broad and changeable range of conditions.

Although Sanz-Moreno et al. (2008) have made a number of important discoveries, their findings also raise additional questions. Given the large number of RhoGEFs and RhoGAPs, it is notable that DOCK3/NEDD9 and ARHGAP22 are sufficient for Rac activation and inactivation, respectively. It remains to be determined whether these proteins also contribute to the movement of other types of tumor cells. RhoA is highly active in the amoeboid mode of movement, but the RhoGEF (or multiple RhoGEFs) responsible has yet to be identified. More challenging will be working out the mechanisms responsible for the inhibition of Rac activity by Rho signaling and vice versa. Although ROCK activation was sufficient to repress Rac activity, this does not appear to work via direct phosphorylation of ARHGAP22 by ROCK; instead, actin-myosin contractility regulates ARHGAP22-mediated inactivation of Rac by unknown means. Similarly, the locus of WAVE2-mediated repression of MLC2 phosphorylation has not been identified; although regulation of RhoA activity seems most likely, it is also possible that the effect occurs directly on MLC2. Given that WAVE2 is a single component of a large multiprotein complex, it will be interesting to determine whether this effect is mediated by the individual protein or the entire WAVE2 complex. It has recently been shown that depletion of the WAVE complex protein Brk1 induces blebbing, suggesting that a functional complex is required for RhoA suppression (Derivery et al., 2008).

These observations also impact upon potential antimetastatic drug therapies—if one mode of motility is targeted, for example by inhibiting protease or ROCK activity, then tumor cells may switch to the other mode. Therefore, it would be desirable to target proteins that may contribute to both modes of movement, for example LIM kinases (Scott and Olson, 2007), to increase the likelihood of successfully blocking the spread of cancer cells.

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Glycogen Synthase Kinase-3 and Cancer: Good Cop, Bad Cop?

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Dogma held that inhibition of the pleiotropic protein kinase glycogen synthase kinase-3 (GSK-3) was procarcinogenic due to its natural repression of β-catenin. Now, Wang et al. have found the reverse in certain leukemias, possibly paving the way for small-molecule GSK-3 inhibitors as selective anticancer agents.

Glycogen synthase kinase-3 (GSK-3) was originally identified and later enshrined as a member of the insulin-signaling pathway responsible for phosphorylation and inactivation of glycogen synthase, a major regulatory enzyme of glycogen metabolism. But it was soon recognized that this kinase paints with far broader strokes, influencing processes that govern cell metabolism, polarity, transcription, cell-cycle division, apoptosis, development, and cell fate. In mammals, GSK-3 exists as two isoforms, encoded by separate genes. GSK-3α (51 kDa) and GSK-3β (47 kDa) are highly conserved and widely expressed kinases that share 98% sequence identity within their catalytic domains (Doble and Woodgett, 2003). While structurally related, these isoforms are not functionally equivalent.

Unlike most protein kinases, GSK-3 is active under resting conditions and is rapidly inhibited by diverse stimuli. For example, insulin, via PI3K/Akt/PKB, induces the inactivation of GSK-3. Many of its cellular targets are held in an inactive state

through inhibitory phosphorylation. Phosphorylation by GSK-3 can also promote the ubiquitination and degradation of target proteins. Dysregulation of GSK-3, or the pathways that control it, has been implicated in various human diseases such as muscle hypertrophy, diabetes, cancer, bipolar mood disorder, schizophrenia, and neurodegenerative diseases (Martinez, 2008).

The therapeutic potential of GSK-3 inhibitors in these disease states has been actively pursued, and several potent chemical inhibitors of GSK-3 have been developed, most of which are ATP competitive and do not discriminate between GSK-3α and GSK-3β. Administration of these inhibitors improves glucose homeostasis and insulin action in rodent models of diabetes and obesity, and there is evidence that the inhibitors may be useful for conditions associated with inflammation such as ischemia, sepsis, and colitis as well as neurodegenerative accumulation of hyperphosphorylated tau (Martinez, 2008). Cumulatively, these data suggest a promising future for GSK-3 antagonists. However, their progress into clinical trials has been clouded by the concern that inhibition of GSK-3 may promote oncogenesis. GSK-3 is a key suppressor of the Wnt, Hedgehog, and Notch pathways that control cellular fate determination and stem cell maintenance. Within these pathways, GSK-3 serves to phosphorylate the pro-oncogenic molecules β-catenin, c-Myc, and c-Jun, targeting them for degradation or inactivation and thereby inhibiting proliferation and self-renewal. However, these pathways are commonly deregulated in human cancers, and furthermore, gain-of-function mutations in these three proteins that interfere with GSK-3 inhibition have been found in cancers of the skin, colon, prostate, and liver (Polakis, 2007). Thus, GSK-3 inhibition could mimic ectopic signaling of these pathways and promote tumorigenesis. However, no direct in vivo evidence has indicated that such a phenomenon occurs upon administration of GSK-3 inhibitors. Rather, recent studies in prostate, pancreatic, and colorectal cancer cell lines indicate that GSK-3 inhibitors lead to significant reduction in cell growth and proliferation (Martinez, 2008; Ougolkov et al., 2006), suggesting that inhibiting GSK-3 may actually be beneficial for the treatment of specific cancers.

In a recent study, Wang et al. (2008) have provided strong evidence demonstrating that GSK-3 activity is essential for maintenance of a subset of leukemias driven by the translocation of the *MLL* (mixed-lineage leukemia) proto-oncogene. *MLL* rearrangements are found in approximately 10% of human leukemias, with >70% frequency of occurrence in infant leukemia. Over 50 different translocation fusion partners have been identified. Patients with *MLL* fusions often have a poor clinical outcome, and thus much effort has focused on seeking new treatments (Krivtsov and Armstrong, 2007).

Using a pharmacological screen, Wang et al. (2008) found that selective inhibitors of GSK-3 (GSK-3 IX, SB216763, and alsterpaullone) specifically inhibited the growth of human *MLL* leukemia but not other leukemia cells. In addition, GSK-3 inhibitors reduced clonogenic potential and proliferation of *MLL*-transduced murine myeloid or B cell progenitors. Importantly, normal primary myeloid progenitors and progenitors transduced by non-*MLL* fusions displayed little sensitivity to drug treatment. The authors demonstrated that this inhibitor effect was indeed mediated by GSK-3, as genetic ablation of GSK-3β and shRNA-mediated knockdown of GSK-3α phenocopied the reduced self-renewing capacity of *MLL*-transformed progenitors in vitro and leukemogenesis in transplanted mice. The Wang et al. study also demonstrated that both GSK-3α and GSK-3β play redundant roles in maintenance of *MLL*-transformed leukemias and that a >75% reduction in kinase activity is required to invoke impairment of leukemogenicity. This resembles the functionally redundant role of GSK-3 within the Wnt pathway in maintaining low cytoplasmic levels of β-catenin in resting cells, such that removal of at least 3 of the 4 *GSK-3* alleles is required for any significant Wnt signaling (Doble et al., 2007). Mechanistically, GSK-3 inhibitors expectedly increased β-catenin levels. While the extent of β-catenin stabilization was similar in both non-*MLL* and human *MLL* leukemia cell lines, the researchers observed that inhibition of GSK-3 induced expression of the cyclin-dependent kinase inhibitor p27Kip1 in only *MLL*-transformed cells. Furthermore, shRNA-p27Kip1 prevented GSK-3 inhibitor-induced growth arrest of *MLL*-leukemic cells (Figure 1).

These findings represent new and contrasting insights into the role of GSK-3 as a negative regulator of proliferation and self-renewal. GSK-3 has been shown to modulate hematopoietic stem cell (HSC) activity in vivo, suggesting that administration of GSK-3 inhibitors may directly enhance the repopulating capacity of transplanted HSCs (Holmes et al., 2008). Recent data indicate that certain leukemias are maintained by a population of self-renewing leukemia stem cells (LSCs) and that they originate from transformed HSCs and committed myeloid progenitors. A major concern is whether LSCs can be specifically targeted without affecting normal HSCs. Wang et al. (2008) provide compelling evidence that inhibitors of GSK-3 could indeed be useful in specifically targeting the LSC population with *MLL* rearrangements without perturbing the normal HSC compartment. There are some interesting questions that arise from their studies. First, why are *MLL*-rearranged leukemia cells, but not other leukemia cells, vulnerable to GSK-3 inhibitors? Wang et al. show that the abundance of the *MLL* fusion oncogenes is not altered by GSK-3 inhibition, but the mechanism by which these cells are sensitized remains unclear, as does the molecular means by which GSK-3 influences p27Kip1. Recently, it was shown that a set of non-*MLL* leukemia cells exhibited overexpression of c-Myc and that the capacity of GSK-3 to associate and target Myc for degradation was reduced. This "GSK-3 resistance" may offer an explanation as to why non-*MLL* cells are insensitive to GSK-3 inhibitors (Malempati et al., 2006). Second, what is the molecular mechanism by which GSK-3 supports *MLL*-induced oncogenesis? The authors allude to the existence of an Akt/GSK-3/p27Kip1 pathway within these cells. They show that a constitutively active Akt mutant suppresses *MLL*-positive cells and this is abolished by coexpression of a GSK-3 mutant that can no longer be phosphorylated and/or inhibited by Akt (Figure 1). It would be of interest to determine whether *MLL*-induced leukemogenesis can occur in GSK-3α/β knockin mice

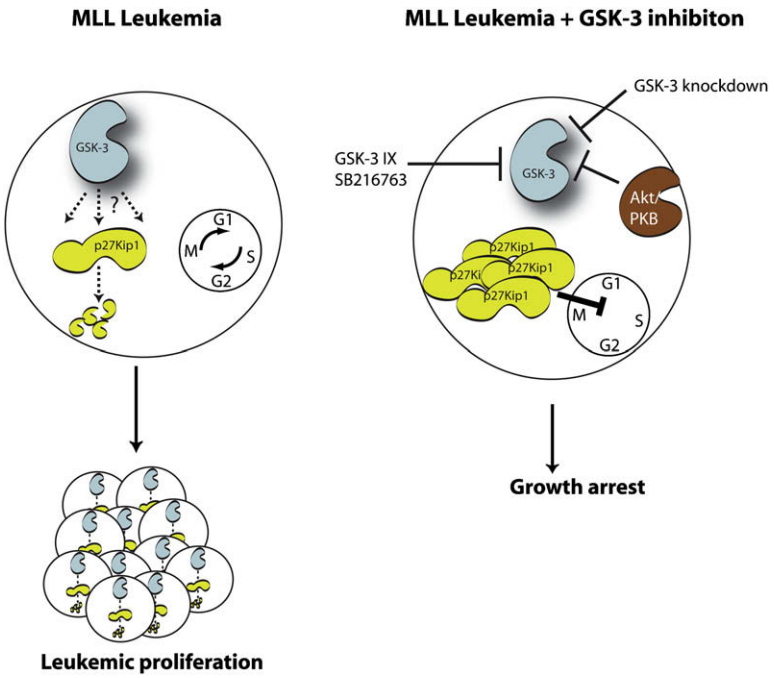

Figure 1. Dependence of *MLL*-Leukemic Cells on GSK-3 Activity for Proliferation
The model of Wang et al. (2008) suggests that GSK-3 supports the maintenance of *MLL* leukemia cells by promoting continuous degradation of the cyclin-dependent kinase inhibitor p27Kip1. Various strategies to inactivate GSK-3 result in stabilization of p27Kip1, which invokes subsequent growth arrest in *MLL* but not non-*MLL* leukemic or normal cells.

expressing non-Akt-inhibitable mutants of GSK-3 (McManus et al., 2005) and, if so, the status of p27Kip1 expression in the tumor cells. Third, and most importantly, what are the long-term effects of high-level GSK-3 inhibition as a therapy for *MLL* leukemias? This new study raises the possibility that activation of p27Kip1 and its growth-inhibitory action will trump pro-oncogenic effects on proteins such as β-catenin. Wang et al. also demonstrated that exposure of animals with *MLL*-like leukemia to lithium, which inhibits GSK-3, prolonged the survival of the treated mice by 40%–50%. For decades, lithium has been widely used for the treatment of bipolar disorder, and there has been no evidence that these patients have increased incidence of cancers. Indeed, mice that have enhanced Wnt signaling and multiple intestinal polyps due to mutation of one copy of *Apc* do not exhibit further increased numbers of polyps when treated with lithium (reviewed in Doble and Woodgett, 2003). Thus, it appears that despite initial concerns that long-term use of GSK-3 inhibitors might promote oncogenesis, evidence is accumulating to suggest that these drugs may in fact be effective in the treatment of certain cancers.

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