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Glycogen Synthase Kinase-3 and Leukemia: Restoring the Balance

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DOI 10.1016/j.ccr.2010.05.017

The role of GSK-3 in oncogenesis is paradoxical, acting as a tumor suppressor in some cancers and potentiating growth in others. In this issue of *Cancer Cell*, Wang et al. provide some mechanistic insight into GSK-3 activity's role in potentiating leukemias which are dependent on *homeobox* (*HOX*) gene misregulation.

Glycogen synthase kinase-3 (GSK-3) was first identified for its role in regulating glycogen metabolism but has since been shown to be involved in the regulation of a variety of processes including signal transduction, gene expression, and cell-fate determination (Jope and Johnson, 2004). These critical roles have become increasingly more appreciated as misregulated GSK-3 has been implicated in neurological disorders (i.e., Alzheimer's disease and bipolar disorder), non-insulin-dependent diabetes mellitus, stroke, and neoplasias (Cohen and Frame, 2001; Rayasam et al., 2009). With over 40 proteins identified as potential GSK-3 substrates, it is not surprising that a complicated network of GSK-3 functioning has emerged (Jope and Johnson, 2004). This complexity is particularly evident in cancer where GSK-3 can take on seemingly opposing roles in tumor suppression or promotion (Ougolkov and Billadeau, 2006; Luo, 2009).

Decreased expression or activity of GSK-3 has been associated with skin and breast tumors. In vitro and in vivo rescue experiments in which active GSK-3 is restored to transformed tumor-cells leads to suppression of cell proliferation. Tumor-suppressive functioning of GSK-3 has been shown to involve the WNT signaling pathway in which active GSK-3 negatively regulates β-catenin through inhibitory phosphorylation and prevents transcription of β-catenin target genes involved in cell-cycle progression (Figure 1). Decreased expression or activity of GSK-3 could, then, activate the WNT signaling pathway through stabilization of β-catenin and contribute to tumorigenesis (Luo, 2009; Rayasam et al., 2009). In contrast, overexpression of active GSK-3 is associated with increased proliferation and decreased patient survival of some cancers through pathways which are thought to involve cyclin D1 and NF-κB (Luo, 2009).

This opposing function of GSK-3 as a tumor promoter has also been suggested for acute leukemia in a report by Wang and colleagues in which MLL-associated leukemia was shown to depend on GSK-3 for sustained proliferation of transformed cells (Wang et al., 2008). Pharmacologic inhibition of GSK-3 caused decreased proliferation, reduced cell-cycle progression, and increased myeloid differentiation of leukemia cells that had been transformed with chimeric MLL oncoproteins. Decreased GSK-3 activity in MLL leukemia cells was associated with increased levels of β-catenin, decreased cell proliferation in vitro, and enhanced survival of mice with these leukemias in vivo. An increase in the CDK inhibitor, $p27^{Kip1}$, expression was observed specifically in MLL leukemia cells upon treatment with GSK-3 inhibitors suggesting that GSK-3 may override cell-cycle regulators in MLL cells to enhance proliferation, though the exact mechanism remained unclear.

In the current issue of *Cancer Cell*, Wang et al. present a follow-up of their previous study where they begin to delineate the mechanism whereby MLL leukemias depend on GSK-3 for maintenance of proliferation and transformation (Wang et al., 2010). Their data demonstrate that GSK-3 activity promotes the formation of a HOX/MEIS1/CREB complex that recruits coactivators CBP and TORC to maintain the MLL leukemia stem cell transcription program. It has been well established that *homeobox* (*HOX*) genes become misregulated in aggressive leukemias involving *MLL* translocations (Shah and Sukumar, 2010). During normal hematopoiesis, MLL maintains appropriate *HOX* gene expression in hematopoietic stem and progenitor cells. As progenitor cells mature into differentiated

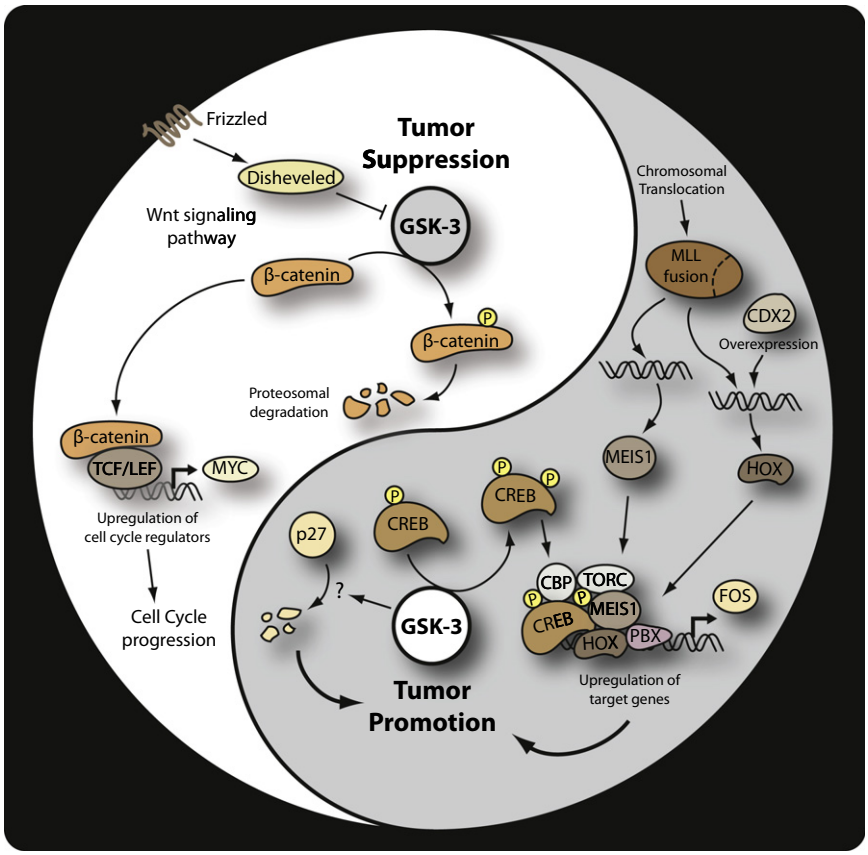

Figure 1. The Seemingly Contrary Roles of GSK-3 Help to Maintain a Fine Balance between Normal Cell-Cycle Progression and Tumorigenesis

Constitutively active GSK-3 acts to destabilize β-catenin through phosphorylation, thus, functioning as a tumor suppressor to limit proliferation (unshaded region). In contrast, GSK-3 promotes expansion of leukemic cells transformed by HOX overexpression through phosphorylation of CREB and subsequent formation of a transcriptional coactivator complex (shaded region).

cells of a committed lineage, the expression of *HOX* cluster genes become downregulated. MLL fusion proteins, on the other hand, cause the aberrant expression of target HOX transcription factors that function in concert with MEIS1 to maintain a transformed capacity in leukemia cells. Wang and colleagues now show that GSK-3 functions downstream of MLL fusion proteins to facilitate HOX-mediated transcription (Figure 1). Pharmacologic inhibition of GSK-3 in HOX-transformed cells (or functional inhibition via conditional expression of AKT) shows decreased proliferation and decreased HOX/MEIS1 transcriptional activity. This inhibitor sensitivity is dependent on the presence of CREB, which is shown to recruit coactivators CBP and TORC to activate transcription of HOX/MEIS1 target genes. The association of this transcriptional activating complex, dependent on GSK-3 serine/threonine kinase activity, ultimately allows for the maintenance of a transformation program which promotes proliferation of the leukemia stem cells.

The identification of a functional role for GSK-3 in MLL-associated leukemia suggests that GSK-3 inhibitors may be a promising therapy that is selective for transformed cells that are dependent on HOX overexpression. It has also been reported that 90% of AML cases have aberrant expression of *caudal-type homeobox transcription factor* (*CDX2*), another regulator of *HOX* genes, further suggesting that proper HOX functioning is critical for maintaining a balance between normal and pathologic states (Rice and Licht, 2007). GSK-3 inhibitors might prove to be particularly efficacious in these *CDX2*-overexpressing leukemias and those with *MLL* translocations that exhibit *HOX* misregulation. In vivo preclinical studies of GSK-3 inhibition with lithium chloride have already shown promise in prolonging survival of mice with HOX-transformed leukemias (Wang et al., 2008, 2010). The lithium ion, though, is a relatively nonspecific inhibitor that competes with magnesium for protein binding, so off-target effects are likely to occur as may also be the case with GSK-3 inhibitors which competitively inhibit ATP binding. Now that a critical complex has been identified as a target for GSK-3 activity in MLL leukemias, more specific substrate inhibitors may be developed that selectively target the critical formation of the HOX/MEIS1/CREB complex. It is also possible that monotherapy with a GSK-3 inhibitor may deplete normal hematopoietic stem cells (HSCs) in addition to targeting leukemic stem cells dependent on GSK-3 activity. A recent report has shown that GSK-3 is necessary for self-renewal of normal HSCs and that knockdown of both α and β GSK-3 isoforms may significantly deplete these normal HSC pools (Huang et al., 2009). Combination therapy with rapamycin may maintain the HSC pool while allowing the GSK-3 inhibitor to deplete the leukemia stem cells for an effective therapy against this aggressive form of leukemia.

It will be important to understand how the tumor-promoting function of GSK-3 is counterbalanced by its tumor suppressor functions in leukemic cells, particularly if GSK-3 inhibitors are used therapeutically, which may ultimately block activity in either capacity. Cells that are dependent on the tumor suppressor actions of GSK-3 could be adversely affected by GSK-3 inhibition. What factors ultimately determine which function of GSK-3 function will predominate in a cell? Can the opposing activities of GSK-3 be utilized to override the HOX-mediated oncogenic program proposed by Wang et al.? A better appreciation of GSK-3 functions in signal transduction, cell-cycle regulation, and cell-fate determination may eventually allow for the effective application of GSK-3 inhibitors in the clinic. Wang et al. provide a novel mechanism by which GSK-3 promotes transformation, and in doing so, the authors provide a new therapeutic strategy which may lead to better clinical outcomes for aggressive MLL-associated leukemias.

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PAF Is in the Cabal of MLL1-Interacting Proteins that Promote Leukemia

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DOI 10.1016/j.ccr.2010.05.019

MLL1 fusions are among the most potent oncogenic drivers of leukemia development. In recent articles published in *Molecular Cell* and in *Cancer Cell*, researchers find that MLL1 fusions are reliant on a physical interaction with the PAF transcription elongation complex for their recruitment to chromatin and, consequently, leukemic transformation.

Acute myeloid or lymphoid leukemias harboring rearrangements of the MLL1 gene represent a poor-prognosis subset of these diseases with a general unresponsiveness to chemotherapy (reviewed in Krivtsov and Armstrong, 2007). Chromosomal translocations that disrupt MLL1 generate oncogenic gene fusions encoding the MLL1 N-terminal region fused to one of a variety of different partner proteins (>50 are known). A consistent feature of otherwise diverse fusion partners is the corruption of MLL1's normal capacity to promote self-renewal of hematopoietic cells. The resulting MLL1 fusion undermines normal differentiation pathways to immortalize hematopoietic cells in an immature state. While evidence from mouse models has established MLL1 fusions as among the most potent drivers of leukemia known, effective strategies have yet to be identified for neutralizing leukemic MLL1 functions for therapeutic benefit. One avenue toward identifying novel therapeutic handles in these aggressive leukemias is to elucidate the essential biochemical framework of MLL1 fusion protein complexes. Two recent articles published in *Cancer Cell* and *Molecular Cell* have made a pivotal advance in this regard by identifying a specific interaction between the PAF complex and MLL1 that is required for leukemic transformation (Milne et al., 2010; Muntean et al., 2010). Hence, PAF is exposed as a conspirator that, along with two other MLL1-associated proteins, Menin and LEDGF, promotes leukemogenesis conferred by MLL1 fusion proteins.

MLL1 performs its normal and leukemic functions through involvement with active chromatin states (Krivtsov and Armstrong, 2007). Like many other chromatin regulators, MLL1 is composed of an assortment of domains (AT hooks, CXXC, BROMO, PHD) that can latch onto DNA or histones, as well as a catalytic SET domain at the C terminus that methylates histone H3 at lysine 4, a modification implicated in active transcription (Milne et al., 2002). MLL1 also has been shown to interact with numerous proteins to form a higher-order complex, e.g., Menin, LEDGF, HCF-1, ASH2L, RbBP5, and WDR5 (Yokoyama et al., 2004). In contrast to the full-length molecule, the MLL1 fragment present in leukemogenic fusions only retains the Menin/LEDGF interaction domain, AT hooks, and the CXXC domain, which together are sufficient for recruitment to target sites in the genome. Distortion of MLL1 function is due to the replacement of its native C-terminal effector domains with those provided by one of many C-terminal fusion partners. Indeed, a large number of studies have identified protein complexes associated with many of the most common MLL1 fusion partners (ENL, AF4, and AF9), all of which seem to share a group of factors linked with regulating transcription elongation, e.g., pTEFb and the histone methyltransferase DOT1L (e.g., Mueller et al., 2007). Thus, MLL1 fusions assemble a multisubunit complex of transcriptional regulators that leads to altered expression of MLL1's normal target genes, such as HOXA9.

A major mechanistic question addressed in the articles by Muntean et al. (2010) and Milne et al. (2010) regards the recruitment mechanism employed by the