Cancer Cell
Article

GSK-3 Promotes Conditional Association of CREB and Its Coactivators with MEIS1 to Facilitate HOX-Mediated Transcription and Oncogenesis

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DOI 10.1016/j.ccr.2010.04.024

SUMMARY

Acute leukemias induced by MLL chimeric oncoproteins are among the subset of cancers distinguished by a paradoxical dependence on GSK-3 kinase activity for sustained proliferation. We demonstrate here that GSK-3 maintains the MLL leukemia stem cell transcriptional program by promoting the conditional association of CREB and its coactivators TORC and CBP with homedomain protein MEIS1, a critical component of the MLL-subordinate program, which in turn facilitates HOX-mediated transcription and transformation. This mechanism also applies to hematopoietic cells transformed by other *HOX* genes, including *CDX2*, which is highly expressed in a majority of acute myeloid leukemias, thus providing a molecular approach based on GSK-3 inhibitory strategies to target HOX-associated transcription in a broad spectrum of leukemias.

INTRODUCTION

Homeobox (*HOX*) genes comprise one of the largest groups of annotated oncogenes (Futreal et al., 2004), and are implicated in the pathogenesis of various human cancers (Abate-Shen, 2002; Sitwala et al., 2008; Svingen and Tonissen, 2003). They encode a large and diverse family of transcription factors that share a conserved 60 amino acid homeodomain DNA-binding motif. Originally discovered through their causative roles in homeotic patterning defects, HOX proteins are critical regulators of cell fate, organ and tissue formation, and stem cell functions. In the blood system, HOX proteins regulate hematopoietic stem cell self-renewal, a process that is perturbed in acute leukemias by either activating mutations of *HOX* genes themselves, or more commonly by mutations or misexpression of their upstream regulators MLL and CDX2, respectively (Dou and Hess, 2008; Liedtke and Cleary, 2009; Riedt et al., 2009; Scholl et al., 2007). Expression of HOXA9 in particular has been linked with the general prognosis of acute myeloid leukemia (AML). The HOX regulatory pathway, therefore, constitutes a potential target for therapeutic interventions in leukemias and other malignancies.

The DNA-binding and transcriptional properties of HOX proteins are enhanced by interactions with TALE (three amino acid loop extension) class homeodomain proteins of the PBX and MEIS families (Owens and Hawley, 2002; Sitwala et al., 2008). Genetic studies reveal that TALE proteins are required for many HOX-dependent developmental and oncogenic programs. Coexpression of MEIS1 with HOXA9 markedly shortens the latency for myeloid leukemia in mouse models (Kroon et al., 1998), and mutations of HOXA9 that prevent interactions with PBX proteins abrogate its oncogenic properties (Schnabel et al., 2000). MEIS1 is consistently expressed at high levels in MLL and CDX2 leukemias, and serves an essential and rate-limiting role in regulating MLL leukemia stem cell potential (Rawat et al., 2008; Wong et al., 2007). TALE proteins form hetero-oligomeric complexes with HOX proteins to recruit a variety of transcriptional coregulators with either coactivator or corepressor properties. PKA signaling has been specifically implicated in the recruitment of coactivators by TALE

Significance

Increasing evidence indicates that inhibition of the GSK-3 multifunctional serine/threonine kinase impairs the proliferation and induces the differentiation of a variety of cancers, including leukemias induced by MLL oncogenes. Conversely, GSK-3 inhibition also stimulates the activities of several oncogenic proteins, therefore it is critical to determine the underlying mechanisms that dictate its biphasic oncogenic properties. In this report, we demonstrate that GSK-3 activity maintains the physical and functional association of CREB and its coactivators with MEIS1, a HOX DNA-binding cofactor and critical downstream mediator of the MLL oncogenic program. This in turn promotes critical target gene expression responsible for HOX-mediated transformation. These findings provide a molecular rationale for targeting HOX-associated transcription through GSK-3 inhibition in a subset of leukemias.

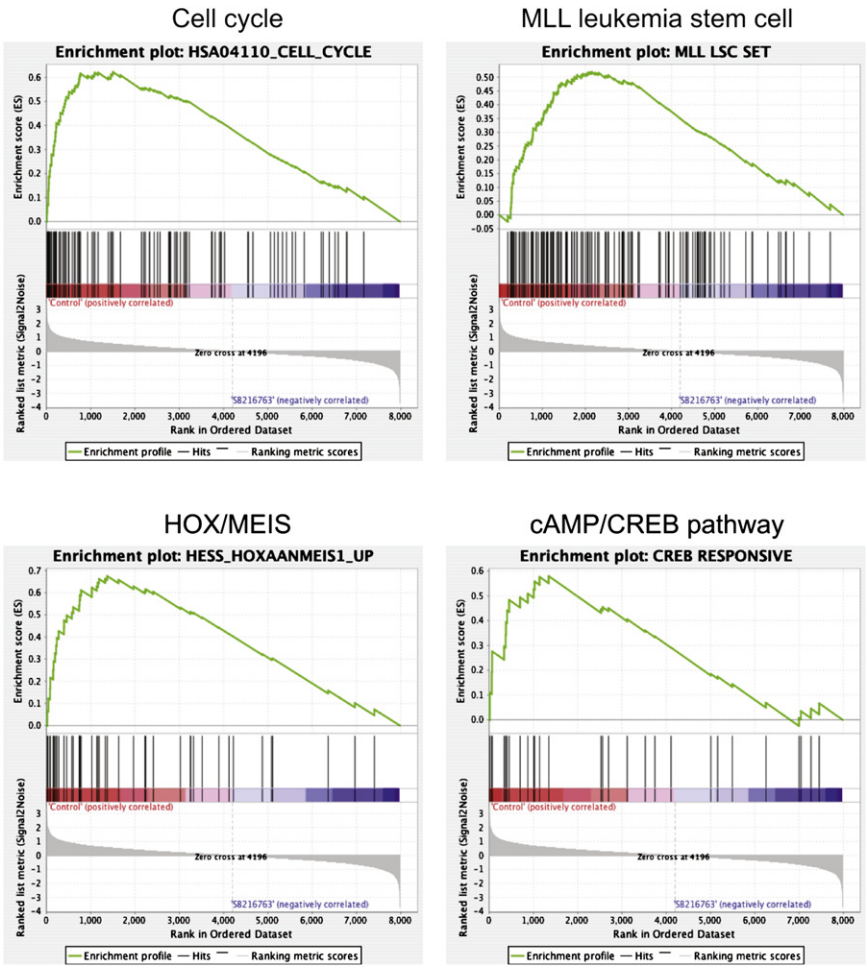

Figure 1. Global Gene Expression Changes of MLL Cells in Response to GSK-3 Inhibition

The data set of gene expression differences resulting from GSK-3 inhibitor treatment (10 μM SB216763 for 20 hr) was used for GSEA. Enrichment plots are shown for selected downregulated gene sets identified by GSEA (Table S2). See also Tables S1 and S2.

proteins, and possibly in the interconversion of coregulator recruitment underlying differential transcriptional activity (Goh et al., 2009; Huang et al., 2005). Despite these advances, the signaling pathways that coordinate HOX-TALE transcriptional outputs in normal and neoplastic cells remain largely undefined.

We have previously shown that glycogen synthase kinase 3 (GSK-3) is required for maintenance of leukemias with MLL mutations (Wang et al., 2008). GSK-3 is a serine/threonine kinase that functions on several signaling pathways implicated in various pathological processes including diabetes, inflammation, and neurodegenerative disorders (Cohen and Goedert, 2004; De Ferrari and Inestrosa, 2000; Doble and Woodgett, 2003; Martin et al., 2005). In malignancies, inactivating mutations of GSK-3 underscore its normal tumor suppressor function to downregulate growth-promoting pathways such as those mediated by WNT, Hedgehog, and MYC proteins that are inappropriately activated in cancers (Cohen and Goedert, 2004). However, increasing evidence demonstrates that GSK-3 serves a tumor-promoting role to sustain proliferation in some cancers, thus opening up the possibility of targeting GSK-3 for therapeutic purposes (Luo, 2009). Defining the underlying mechanisms that mediate GSK-3 dependence of specific tumors will provide a molecular rationale for selective application of therapies that target GSK-3. In this report, we investigate how GSK-3 facilitates HOX-mediated transcription and oncogenesis.

RESULTS

GSK-3 Maintains the MLL Leukemia Stem Cell Transcriptional Program

Gene expression profiling was performed to investigate the mechanisms underlying MLL leukemia dependence on GSK-3. Following GSK-3 inhibitor (SB216763) treatment of the RS4;11 human leukemia cell line, which contains an MLL-AF4 chromosomal translocation, 1028 differentially expressed genes were identified, of which 554 were upregulated and 474 downregulated at least 1.5-fold (see Table S1 available online). Comparison of the treatment data set with curated gene sets derived from diverse published experiments (Subramanian et al., 2005) revealed that downregulated genes were significantly enriched for gene sets related to cell cycle (Figure 1), as well as MYC-regulated and differentiation-associated genes (Table S2), consistent with growth arrest, decreased MYC expression, and differentiation changes in MLL myeloid leukemia cells upon GSK-3 inhibition (Wang et al., 2008).

Genes comprising the MLL leukemia stem cell (LSC) maintenance program, which are shared with embryonic stem cells as well as poor prognosis human cancers (Somervaille et al., 2009), were significantly downregulated (Figure 1 and Table S2), indicating that GSK-3 likely affects MLL LSC potential. Downregulated genes were also significantly enriched for gene sets associated with HOX overexpression, including those induced by coexpression of HOXA9 and MEIS1 (Figure 1), which are direct MLL transcriptional targets implicated in leukemia pathogenesis. Furthermore, MYB, a downstream mediator of HOXA9/MEIS1 in AML (Hess et al., 2006), and its subordinate transcriptional program, were also downregulated (Table S2). Thus, GSK-3 inhibition appears to target the LSC program at or near the apex of the transcriptional hierarchy initiated by MLL oncoproteins.