Chromatin

Major finding: BRD4-mediated transactivation activity in AML depends upon its interaction with NSD3-short.

Mechanism: NSD3-short links BRD4 and CHD8 at active promoters and super-enhancer regions in AML.

Impact: BET inhibitors act by removing BRD4–NSD3–CHD8 complexes from chromatin to suppress transcription.

THE SHORT ISOFORM OF NSD3 COUPLES BRD4 AND CHD8 TO DRIVE TRANSCRIPTION

Members of the bromodomain and extraterminal (BET) family of transcriptional coactivators, which bind to acetyl-lysine motifs on nuclear proteins via tandem bromodomain interaction modules, have been implicated as therapeutic targets in different cancers. The BET family member bromodomain-containing 4 (BRD4) is necessary for the expression of driver oncogenes such as *MYC* in hematologic cancers and thus is a therapeutic target, particularly in acute myeloid leukemia (AML). To identify the effector proteins required for BRD4-mediated transcription, Shen and colleagues investigated the interaction between BRD4 and two splice isoforms of the histone H3 lysine 36 methyltransferase nuclear SET domain–containing protein 3 (NSD3, encoded by *WHSC1L1*), NSD3-long and NSD3-short, in AML. Immunoprecipitation experiments revealed that BRD4 interacts with both NSD3 isoforms, but knockdown and rescue experiments showed that only NSD3-short was essential for AML proliferation. NSD3-short, which lacks lysine methyltransferase activity, interacted with the BRD4 ET domain at residues required for BRD4-mediated transcriptional activation, indicating that NSD3-short acts as an adaptor for BRD4. Consistent with this finding, immunoprecipitation and mutagenesis studies showed that BRD4 and the chromatin remodeling ATPase chromodomain helicase DNA-binding protein 8 (CHD8) both interacted with NSD3-short, which was necessary for the association of CHD8 with the BRD4 ET domain. NSD3-short contained four independent interaction surfaces that were required for AML proliferation: an N-terminal acidic transactivation domain, the BRD4-interacting region, a PWWP chromatin reader module, and the CHD8-interacting region. Similar to BRD4 inhibition, knockdown of NSD3-short or CHD8 induced differentiation of AML cells and loss of BRD4-mediated gene expression. Furthermore, chromatin immunoprecipitation experiments revealed that BRD4, NSD3, and CHD8 colocalized across the AML genome and BRD4 inhibition induced NSD3 and CHD8 release from super-enhancer regions. Together, these results elucidate the roles of NSD3-short and CHD8 in BRD4-mediated transcriptional activation and provide a rationale for therapeutically targeting NSD3-short and CHD8. ■

Shen C, Ipsaro JJ, Shi J, Milazzo JP, Wang E, Roe JS, et al. NSD3-short is an adaptor protein that couples BRD4 to the CHD8 chromatin remodeler. Mol Cell 2015 Nov 25 [Epub ahead of print].

Transcriptional Regulation

Major finding: The *Khps1* lncRNA facilitates expression of *SPHK1* via chromatin remodeling and E2F1 recruitment.

Mechanism: *Khps1* forms a DNA–RNA triplex structure at a homopurine stretch in the *SPHK1* promoter.

Impact: LncRNAs can promote tumorigenesis through upregulation of proto-oncogenes such as *SPHK1*.

Khps1 LncRNA PROMOTES E2F1-DEPENDENT EXPRESSION OF *SPHK1*

Thousands of long noncoding RNAs (lncRNA) have been identified; however, little is known about their functions. It has been proposed that lncRNAs can form DNA–RNA triplexes at homopurine stretches in gene regulatory elements, which might direct targeted recruitment of epigenetic regulators and thereby alter gene expression. To test the hypothesis that lncRNAs mediate epigenetic gene regulation through DNA–RNA triplex structures, Postepska-Igielska and colleagues studied the sphingosine kinase 1 (*SPHK1*) gene, a proto-oncogene with multiple triplex-forming regions upstream of the transcription start site. RNA sequencing identified a lncRNA, *Khps1*, transcribed in the antisense orientation from the *SPHK1* locus. The *Khps1* promoter contained putative E2F transcription factor binding sites, which were confirmed by chromatin immunoprecipitation. *SPHK1* transcription required *Khps1* expression, which correlated with E2F1 occupancy at the *SPHK1* promoter, and E2F1 promoted transcription of both the sense and antisense transcripts, with a more rapid increase in *Khps1* expression. Knockdown of *Khps1* resulted in reduced E2F1 occupancy at the *SPHK1* promoter, but not a control promoter, and prevented E2F1-mediated induction of *SPHK1*, suggesting that *Khps1* promotes *SPHK1* transcription by facilitating E2F1 binding to the *SPHK1* promoter. Consistent with this idea, *Khps1* produced an active chromatin structure at the *SPHK1* promoter through recruitment of the histone acetyltransferase p300/CBP, which resulted in an increase in activating histone marks and reduced nucleosome density that facilitated E2F1 binding. *Khps1* interacted directly with the *SPHK1* promoter, forming a DNA–RNA triplex requiring Hoogsteen base pairing at a homopurine stretch upstream of the *SPHK1* transcription start site. Expression of both *Khps1* and SPHK1 was highest during the G1/S transition of the cell cycle, promoting cell growth and lessening the proapoptotic effects of E2F1. Together, these results illustrate that the lncRNA *Khps1* promotes expression of the proto-oncogene *SPHK1* via DNA–RNA triplex formation and chromatin remodeling. ■

Postepska-Igielska A, Giwojna A, Gasri-Plotnitsky L, Schmitt N, Dold A, Ginsberg D, et al. LncRNA Khps1 *regulates expression of the proto-oncogene* SPHK1 *via triplex-mediated changes in chromatin structure. Mol Cell 2015;60:626–36.*