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POSTER ABSTRACTS

604.MOLECULAR PHARMACOLOGY AND DRUG RESISTANCE: MYELOID NEOPLASMS

Differentiation of Acute Myeloid Leukemia Cells upon Pharmacological Inhibition of LSD1 Requires Its N-Terminal Intrinsically Disordered Region

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Lysine-specific histone demethylase 1 (LSD1/KDM1A) governs hematopoiesis by regulating hematopoietic stem cell renewal and proper differentiation. Additionally, LSD1 is required for the maintenance of acute myeloid leukemia (AML) cells and has emerged as a key therapeutic target. Prior work from our group and others have revealed that the LSD1 demethylase activity is not required for AML proliferation. Instead, the interaction between LSD1 and GFI1/GFI1B is necessary to sustain AML cell survival. Thus, beyond its canonical demethylase activity, LSD1 has nonenzymatic, transcription factor (TF)-specific scaffolding functions critical for its function. Most LSD1 inhibitors function by blocking protein-protein interactions between LSD1 and TFs, even though they were originally designed as enzyme inhibitors. While studies indicate that LSD1 has important scaffolding functions, how LSD1 can coordinate with TFs to control gene expression programs is not well understood.

Here, we used CRISPR-suppressor scanning to identify regions of LSD1 outside the active site that when mutated can lead to resistance to the covalent LSD1 inhibitor, GSK-LSD1. The understudied N-terminal intrinsically disordered region (IDR) of LSD1 was among the most enriched regions. To explore this further, we generated clonal AML cell lines harboring in-frame deletions within the IDR. These IDR-mutant cell lines were drug resistant and failed to differentiate upon GSK-LSD1 treatment. Moreover, knockdown of endogenous LSD1 followed by overexpression of LSD1 IDR-mutants rescued growth in the presence of GSK-LSD1, confirming that a fully functional IDR is necessary for differentiation.

We performed LSD1 co-immunoprecipitation followed by mass spectrometry in wild-type and IDR-mutant cells to measure changes in LSD1 interactors upon GSK-LSD1 treatment. GSK-LSD1 treatment disrupted the LSD1-GFI1B interaction in both wild-type and mutant cells at comparable levels. Since GSK-LSD1 still induces dissociation of the mutant complex but has minimal effect on proliferation, our results suggest that the IDR-mutant cells no longer require GFI1B. In support, knockout of GFI1B inhibited growth of wild-type but not IDR-mutant cells. Strikingly, GSK-LSD1 treatment promoted LSD1's interaction with several master regulator TFs in hematopoiesis, including C/EBPα, PU.1, and RUNX1. Notably, C/EBPα was the most up-regulated interactor in both cell lines, with modest enrichment in IDR-mutant over wild-type. Using live-cell imaging assays, we found that deletions within the LSD1 IDR enhance association with these TFs. These results suggest that GSK-LSD1 reprograms LSD1-TF interactions, disrupting LSD1-GFI1B to induce LSD1-C/EBPα association, and that the IDR mutations alters this LSD1 redistribution to other TF partners.

LSD1 is known to silence gene enhancers during differentiation in a process termed "enhancer decommissioning". The mechanisms by which LSD1 occupies and controls enhancers remain elusive. To test if the LSD1 IDR regulates enhancer elements, we profiled LSD1 and H3K27ac using ChIP-seq in wild-type and IDR-mutant cells upon GSK-LSD1 treatment. Upon GSK-LSD1 treatment, disruption of LSD1-GFI1B binding was accompanied by increased LSD1 levels at other TF and H3K27ac sites, supporting the notion that loss of LSD1-GFI1B interaction induces LSD1 redistribution to other TF partner sites. A concomitant increase in H3K27ac levels was observed across LSD1 sites co-bound by C/EBPα, PU.1, or RUNX1 in wild-type cells. By contrast, in IDR-mutant cells, increases in H3K27ac levels were blocked at these sites upon GSK-LSD1 treatment, suggesting that full enhancer activation is impaired by mutations in the IDR, potentially due to altered association with master regulator TFs. This points to a function of LSD1 IDR in controlling enhancer activation for differentiation.

Collectively, our study reveals a role for IDR in regulating LSD1-TF interactions, controlling enhancer activation that is necessary for AML cell differentiation. These data provide deep functional and mechanistic insights into the role of LSD1 IDR and offer important considerations for pharmacological targeting of protein-protein interactions.

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