Signaling

Major finding: Additional NOTCH1 cofactors beyond CSL and MAML are required for NOTCH1 target activation.

Concept: LSD1 and PHF8 histone demethylase activity is required for expression of NOTCH1 target genes.

Impact: Histone demethylase–targeted therapies may be effective in Notch-dependent cancers.

A MULTIFUNCTIONAL COMPLEX REGULATES NOTCH1 NUCLEAR ACTIVITY

The Notch signaling pathway is a key determinant of developmental programs and is deregulated in human cancers. Upon ligand binding, Notch receptors undergo proteolytic cleavage and the intracellular domain of Notch (ICN) translocates to the nucleus, where it converts CSL from a repressor to an activator and forms a complex with CSL and Mastermind-like (MAML) coactivators to induce target gene expression. To gain insight into the regulation and function of the ICN–CSL–MAML complex, Yatim and colleagues purified epitope-tagged ICN and identified interacting proteins by mass spectrometry. As expected, CSL and MAML1 were stoichiometric binding partners, but over 100 other NOTCH1-interacting proteins were also identified. ICN, CSL, and MAML were found to assemble into a complex with the transcriptional activator AF4p12, the histone demethylases LSD1 and PHF8, and the SWI/SNF nucleosome remodeling complex subunits BRG1 and PB1, all of which were required for expression of Notch target genes. In the presence of ICN, PHF8 removed the repressive histone H3 lysine-27 dimethylation (H3K27me2) mark and LSD1 removed the repressive H3K9me2 mark from Notch targets to promote gene expression. However, LSD1 was also associated with the CSL repressor in the absence of ICN and contributed to CSL-mediated Notch target repression by removing the activating H3K4me2 mark, suggesting that LSD1 plays a dual role in Notch signaling regulation. To determine whether histone demethylases play a role in oncogenic Notch signaling, the authors knocked down PHF8 and LSD1 in *NOTCH1*-mutant T-cell acute lymphoblastic leukemia (T-ALL) cell lines and observed decreased cell proliferation and complete abrogation of T-ALL xenograft tumor growth. These data thus underscore the importance of histone demethylases in transcriptional regulation of Notch target genes and implicate PHF8 and LSD1 as potential therapeutic targets in Notch-dependent cancers. ■

Yatim A, Benne C, Sobhian B, Laurent-Chabalier S, Deas O, Judde JG, et al. NOTCH1 nuclear interactome reveals key regulators of its transcriptional activity and oncogenic function. Mol Cell 2012 Sept 27 [Epub ahead of print].

Melanoma

Major finding: NRAS-driven biologic outputs are gated and can be blocked by combined MEK and CDK4 inhibition.

Approach: Global network modeling was used to identify cooperative strategies that mimic NRAS inhibition.

Impact: This therapeutic approach may provide clinical benefit in *NRAS*-mutant melanoma.

NETWORK ANALYSIS OF NRAS ACTIVITY DEFINES A SYNERGISTIC DRUG STRATEGY

NRAS mutations are common in melanoma, leading to enhanced downstream activation of the MAPK cascade. However, the use of MEK inhibitors alone has limited efficacy in these tumors, and attempts to directly target oncogenic RAS activity have thus far been unsuccessful. To establish improved drug combinations that mimic RAS inhibition, Kwong and colleagues used an inducible genetically engineered mouse model, in which melanoma formation is dependent on sustained mutant $NRAS^{Q61K}$ expression, to analyze the effects of perturbations on the oncogenic NRAS signaling network. In contrast with withdrawal of mutant *NRAS* expression, which resulted in tumor regression, treatment with pharmacologic MEK inhibitors was only sufficient to induce tumor stasis, suggesting that these drugs do not completely block NRAS-dependent signaling. Consistent with this idea, RAS inhibition via elimination of $NRAS^{Q61K}$ expression triggered apoptosis and diminished mitotic activity in mutant *NRAS*-driven tumors, whereas MEK inhibition only induced apoptosis, indicating that cell-cycle arrest is also required for tumor regression. Furthermore, comparison of global expression profiles and knowledge-based pathway analysis showed an enrichment of cell-cycle regulatory pathways, including activation of the Rb checkpoint, upon $NRAS^{Q61K}$ withdrawal. Network modeling of gene–pathway transcriptional relationships identified cyclin-dependent kinase 4 (CDK4) as a critical mediator of this cell-cycle checkpoint, suggesting that CDK4 inhibition may be clinically beneficial. Indeed, CDK4 blockade synergized with a MEK inhibitor to promote both cell-cycle arrest and apoptosis and to induce tumor regression in mouse and human xenograft models. In addition, partial inhibition of NRAS–MAPK signaling at an earlier time point after $NRAS^{Q61K}$ withdrawal stimulated apoptosis but not cell-cycle arrest, supporting a gradient model of NRAS signaling in which biologic outputs are gated at distinct thresholds. These findings underscore the usefulness of systems biology approaches for developing nonobvious combinatorial therapeutic strategies. ■

Kwong LN, Costello JC, Liu H, Jiang S, Helms TL, Langsdorf AE, et al. Oncogenic NRAS signaling differentially regulates survival and proliferation in melanoma. Nat Med 2012;18:1503–10.

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