Published OnlineFirst January 6, 2020; DOI: 10.1158/2159-8290.CD-RW2020-001

Leukemia

Major Finding: HBO1 is required for maintenance of leukemia stem cells (LSC) in acute myeloid leukemia (AML).

Concept: A small-molecule HBO1 inhibitor was effective in AML cell lines and primary human AML cells.

Impact: Further efforts to target HBO1 in AML may lead to therapies that eliminate disease-driving LSCs.

HBO1 IS A TARGETABLE DRIVER OF LEUKEMIA STEM CELL MAINTENANCE

The efficacy of treatments for acute myeloid leukemia (AML) is limited by their inability to completely eliminate leukemia stem cells (LSC). MacPherson and colleagues identified the MYST-family acetyltransferase HBO1 as a key dependency in LSCs and showed that acetylation of histone H3 at lysine residue 14 is the predominant nonredundant chromatin modification made by HBO1. *Ex vivo* experiments revealed that loss of HBO1 was associated with increased apoptosis, cell-cycle arrest at the G_0–G_1 transition, and differentiation of LSCs. Corresponding results were obtained in human AML cell lines in which *HBO1* was deleted. Data confirming the critical role of HBO1 in LSC maintenance were obtained using mouse models: *Hbo1*-knockdown LSCs were unable to perpetuate leukemia in mice, and experiments in which *Hbo1* was conditionally deleted provided complementary supporting evidence. Many genes were downregulated by loss of HBO1; notably, some were homeobox genes, which have established roles in LSC maintenance and are associated with poor prognosis when upregulated in AML. These results combined with additional supporting experimental data clearly identified HBO1 as a potential therapeutic target in AML; however, the development of small-molecule inhibitors of histone acetyltransferases has historically been limited by a lack of selectivity. Using a recently developed chemical scaffold that serves as a platform to create inhibitors of MYST-family acetyltransferases, the group generated WM-3835, a cell-permeable small molecule that inhibits HBO1 more strongly than it does other MYST-family proteins. WM-3835 treatment reduced cell viability and phenocopied loss of HBO1 in numerous AML cell lines and decreased clonogenic potential in primary human AML cells harboring disparate driver mutations. Although the rapid metabolism of WM-3835 precluded *in vivo* experiments, these results highlight the potential of HBO1 inhibition to eradicate LSCs and support the continued investigation of HBO1 as a target in AML treatment. ■

MacPherson L, Anokye J, Yeung MM, Lam EYN, Chan YC, Weng CF, et al. HBO1 is required for the maintenance of leukaemia stem cells. Nature 2020;577:266–70.

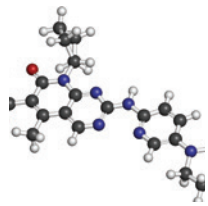
Structural Biology

Major Finding: Structural and biochemical studies revealed how p27 interacts with and activates CDK4.

Concept: p27 binding prevented inhibition of CDK4 by palbociclib, a CDK4/6 inhibitor used in the clinic.

Impact: Palbociclib may not work as previously envisioned, and p27's role in CDK4 regulation is complex.

p27 ACTIVATES CDK4 AND BLOCKS PALBOCICLIB-MEDIATED CDK4 INHIBITION



Some CDK4/6 inhibitors are approved for the treatment of HER2-negative, ER-positive breast cancer and are being investigated for the treatment of other cancers in clinical trials; however, questions remain about CDK4 regulation. Guiley and colleagues investigated CDK4 regulation by the intrinsically disordered proteins p21 and p27, which have seemingly contradictory roles in CDK4 regulation: Some evidence shows that they are CDK inhibitors, but other evidence indicates that they are required for CDK4–cyclin D assembly and activity *in vivo*. Crystal structures of the p21–CDK4–cyclin D1 and p27–CDK4–cyclin D1 trimer complexes revealed that p21's and p27's inhibitory functions may arise from their blockage of a substrate-docking site on cyclin D and the fact that interactions with either one's D2 domains shifts some amino-acid residues that form part of CDK4's ATP-binding pocket. Interestingly, though, comparison of these trimer structures with the structure of the previously published CDK4–cyclin D dimer complex revealed that binding of p21 or p27 caused rotation of the N-lobe of CDK4 relative to the C-lobe, releasing the activation segment of the protein from the active site, and caused a rearrangement that poised the catalytic lysine residue for activity. Thus, while still inhibited, the trimer conformations may represent intermediates along the pathway to activation. Biochemical studies showed that tyrosine phosphorylation of p27, but not p21, led to increased RB phosphorylation by CDK4, and a structure of the phospho-p27–CDK4–cyclin D complex indicated that this effect was due to relief from inhibition by p27's D2 domain, which binds CDK4's N-lobe. Notably, the CDK4/6 inhibitor palbociclib could not bind p27–CDK4–cyclin D trimers and poorly inhibited these trimers both *in vitro* and in a breast cancer cell line. Instead, palbociclib primarily bound CDK4/6 monomers in cells, and treatment appeared to indirectly cause CDK2 inhibition, indicating that its mechanism may be different than what is generally thought. In summary, this study provides mechanistic insight into a process important in cancer and sheds light on a potentially misunderstood drug mechanism. ■

Guiley KZ, Stevenson JW, Lou K, Barkovich KJ, Kumarasamy V, Wijeratne TU, et al. p27 allosterically activates cyclin-dependent kinase 4 and antagonizes palbociclib inhibition. Science 2019;366. pii:eaaw2106.

AACR American Association for Cancer Research

CANCER DISCOVERY

p27 Activates CDK4 and Blocks Palbociclib-Mediated CDK4 Inhibition

Cancer Discov 2020;10:172. Published OnlineFirst January 6, 2020.