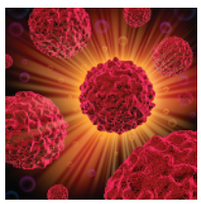
Drug Resistance

Major finding: Mutations in the *de novo* purine biosynthesis enzyme PRPS1 drive thiopurine resistance in relapsed ALL.

Mechanism: PRPS1 mutants lose negative feedback of purine biosynthesis and inhibit prodrug activation.

Impact: Inhibitors of purine biosynthesis may overcome thiopurine resistance in patients with relapsed ALL.

THIOPURINE RESISTANCE IN CHILDHOOD ALL IS MEDIATED BY *PRPS1* MUTATIONS



The thiopurines 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG) are prodrugs that are converted to cytotoxic purine antimetabolites and administered as part of combination chemotherapy in acute lymphoblastic leukemia (ALL). Thiopurine resistance contributes to ALL relapse, a leading cause of mortality in childhood cancers. To investigate how genetic mutations contribute to ALL relapse, Li, Li, Bai, and colleagues performed whole-exome sequencing of 16 matched samples obtained at diagnosis, remission, and relapse from children with ALL, and identified relapse-specific somatic mutations in phosphoribosyl pyrophosphate synthetase 1 (*PRPS1*), which encodes an enzyme essential for purine biosynthesis. *PRPS1* mutations were confirmed in 24 (6.7%) of 358 relapsed B-cell ALL cases in validation cohorts and were associated with early disease relapse. Ultra-deep sequencing of serial bone marrow samples demonstrated that *PRPS1* mutations were not present at diagnosis and increased exponentially before clinical relapse. Expression of gain-of-function PRPS1 mutants resulted in increased viability and resistance to apoptosis after treatment with 6-MP and 6-TG, confirming that PRPS1 confers thiopurine resistance. PRPS1 drug-resistant mutants exhibited reduced nucleotide feedback inhibition of PRPS1 activity, which allowed for continued activation of *de novo* purine biosynthesis despite elevated intracellular nucleotide concentrations. In addition, enhanced *de novo* purine biosynthesis resulted in increased levels of the metabolite hypoxanthine, which competitively inhibited 6-MP conversion. Importantly, knockdown of genes that encode for *de novo* pathway–specific enzymes or treatment with lometrexol, a small-molecule inhibitor of *de novo* purine biosynthesis that is in clinical development, reversed thiopurine drug resistance in *PRPS1*-mutant cells. Overall, these findings demonstrate that *PRPS1* mutations can drive thiopurine resistance via defective negative feedback of nucleotide biosynthesis and competitive inhibition of prodrug activation and suggest that chemotherapeutic agents that inhibit *de novo* purine synthesis may effectively overcome thiopurine resistance in relapsed childhood ALL. ■

Li B, Li H, Bai Y, Kirschner-Schwabe R, Yang JJ, Chen Y, et al. Negative feedback–defective PRPS1 mutants drive thiopurine resistance in relapsed childhood ALL. Nat Med 2015;21:563–71.

Drug Discovery

Major finding: Targeting protein domain-encoding exons in CRISPR–Cas9 screens can reveal genetic dependencies.

Concept: Protein domain functional importance may be inferred from negative selection in a CRISPR–Cas9 screen.

Impact: The identification of protein domains required for cancer cell survival may guide drug target discovery.

DOMAIN-FOCUSED CRISPR SCREENING IDENTIFIES POTENTIAL DRUG TARGETS

CRISPR–Cas9-mediated genome editing has emerged as a useful tool to generate gene-specific knockouts. Genetic screens using the CRISPR–Cas9 technology can be performed using a library of single guide RNAs (sgRNA) that target the Cas9 endonuclease to specific loci, but sgRNA libraries typically target only 5′ coding exons and may not always cause a phenotype, particularly if functional in-frame variants are produced. In their efforts to use CRISPR–Cas9-induced mutagenesis to identify essential genes in a murine acute myeloid leukemia (AML) cell line, Shi and colleagues observed that the degree of negative selection varied greatly among sgRNAs targeting the same gene. For example, severe negative-selection phenotypes were restricted to sgRNAs targeting the sequence within *Brd4* that encodes the bromodomains or the sequence within *Smarca4* that encodes the ATPase domain. Targeting catalytic domains of other essential genes also led to greater negative selection than targeting 5′ coding exons. Hypothesizing that a negative-selection CRISPR–Cas9 screening strategy exclusively targeting sequences encoding protein domains could identify those required for AML cell growth and survival, the authors designed an sgRNA library that specifically targeted sequences encoding chromatin regulatory domains. Of 192 domains targeted, strong negative selection was observed for 25 domains, many of which had not previously been identified as essential in leukemic cells. A negative-selection screening strategy using sgRNA libraries designed to target potentially druggable protein domains may thus help guide identification and prioritization of drug discovery efforts. ■

Shi J, Wang E, Milazzo JP, Wang Z, Kinney JB, Vakoc CR. Discovery of cancer drug targets by CRISPR-Cas9 screening of protein domains. Nat Biotechnol 2015;33:661–7.

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