DNA Repair

Major finding: Accumulation of nuclear p62, driven by loss of autophagy, decreases chromatin ubiquitination.

Mechanism: p62 inhibits RNF168 ligase activity to prevent the accumulation of DNA repair proteins at DSBs.

Impact: Selective autophagic degradation of p62 is critical for chromatin ubiquitination and DSB repair.

NUCLEAR p62 INHIBITS THE DNA DAMAGE RESPONSE

Recent studies have suggested that selective autophagy and the ubiquitin–proteasome system, which are the major protein degradation pathways in eukaryotic cells, participate in DNA double-strand break (DSB) repair. Further, selective autophagy of dysfunctional protein aggregates requires ubiquitination and the autophagic substrate and cargo receptor p62/sequestosome 1 (SQSTM1), which binds to ubiquitinated components in the cytosol, providing evidence of cross-talk between selective autophagy and the ubiquitin–proteasome system. To elucidate the mechanism underlying the role of autophagy in DSB repair and determine whether this mechanism is ubiquitination-dependent, Wang and colleagues assessed the effects of p62 on chromatin ubiquitination. Inhibition of autophagy resulted in the nuclear accumulation of p62, which subsequently inhibited chromatin and histone ubiquitination induced by ionizing radiation (IR)–mediated DNA damage. Nuclear p62 bound to the motif interacting with ubiquitin domain 1 (MIU1) domain in the E3 ligase ring finger protein 168 (RFN168), which has been implicated in DNA damage–induced ubiquitination, and inhibited RFN168 E3 ligase activity to prevent RNF168-mediated chromatin and histone ubiquitination. Binding of p62 to RNF168 inhibited the RNF168-mediated recruitment of homologous recombination (HR)–related DNA repair proteins—BRCA1, ubiquitin interaction motif containing 1 (RAP80, encoded by *UIMC1*), and RAD51—to DSBs, resulting in decreased HR-mediated DSB repair. Nuclear p62-expressing cells treated with IR exhibited impaired DNA repair kinetics and decreased growth *in vitro* and *in vivo* compared to p62-knockout cells or cells expressing cytoplasmic p62. Taken together, these results identify the mechanism underlying selective autophagy–driven DNA repair and show that nuclear p62 is critical for regulating DSB repair. ■

Wang Y, Zhang N, Zhang L, Li R, Fu W, Ma K, et al. Autophagy regulates chromatin ubiquitination in DNA damage response through elimination of SQSTM1/p62. Mol Cell 2016;63:34–48.

Leukemia

Major finding: Mutant *DNMT3A* promotes leukemogenesis via aberrant epigenetic induction of stem-cell genes.

Concept: DOT1L inhibition reverses mutant DNMT3A–induced gene expression patterns, suppressing leukemogenesis.

Impact: DOT1L targeting may provide therapeutic benefit in patients with *DNMT3A*-mutated leukemia.

TARGETING DOT1L MAY REVERSE LEUKEMOGENIC EFFECTS OF MUTANT DNMT3A

Mutations in DNA methyltransferase 3A (DNMT3A), most commonly *DNMT3A*^{R882H}, occur frequently in acute myeloid leukemia (AML), often together with other mutations. To gain insight into the mechanism by which mutant DNMT3A promotes leukemia and whether mutant DNMT3A cooperates with secondary mutations to induce leukemogenesis, Lu and colleagues transplanted mice with hematopoietic stem/progenitor cells (HSPC) expressing wild-type DNMT3A or DNMT3A^{R882H} with NRAS^{G12D}. DNMT3A^{R882H} accelerated leukemogenesis in the presence of NRAS^{G12D}, whereas DNMT3A^{R882H} did not induce disease and wild-type DNMT3A suppressed leukemogenesis. Additionally, DNMT3A^{R882H} enhanced the ability of progenitor cells to be serially transplanted *in vivo*, indicative of increased leukemia-initiating stem cell (LSC) characteristics, and increased expression of stemness genes. Chromatin immunoprecipitation sequencing indicated that DNMT3A^{R882H} binding was enhanced at enhancers and at CpG dinucleotides. DNMT3A^{R882H} led to hypomethylation of CpGs at cis-regulatory sites of essential stemness genes, and increased H3K27 acetylation, an activating histone mark, at gene-regulatory elements, altogether providing a mechanism by which DNMT3A^{R882H} may activate enhancers and promote expression of AML-associated genes. Similar hypomethylation patterns were observed in human patients with *DNMT3A*^{R882H} AML. To potentially reverse the defects of DNMT3A^{R882H}, inhibitors of epigenetic regulators were screened, and *DNMT3A*^{R882H};*NRAS*^{G12D} LSCs exhibited enhanced sensitivity to an inhibitor of the H3K79 methyltransferase DOT1L. DOT1L inhibition reduced expression of DNMT3A^{R882H}-induced stemness genes, suppressed the growth of DNMT3A^{R882H} AML cells, and delayed AML progression *in vivo*. Consistent with these findings, Rau and colleagues also identified DOT1L as a critical mediator of *DNMT3A*-mutant AML. *Dnmt3a*$^{-/-}$ HPSCs exhibited increased DOT1L expression and H3K79 methylation, and DOT1L-induced H3K79 methylation was enhanced in *DNMT3A*-mutant AML cells. Further, DOT1L inhibition reduced colony-forming ability and induced differentiation of primary *DNMT3A*-mutant AML cells, and inhibited tumor growth in a rat AML xenograft model. Together, these studies define the role of mutant DNMT3A in leukemogenesis and suggest that pharmacologic targeting of DOT1L may impair leukemogenesis in *DNMT3A*-mutant AML. ■

Lu R, Wang P, Parton T, Zhou Y, Chrysovergis K, Rockowitz S, et al. Epigenetic perturbations by Arg882-mutated DNMT3A potentiate aberrant stem cell gene-expression program and acute leukemia development. Cancer Cell 2016;30:92–107.

Rau RE, Rodriguez B, Luo M, Jeong M, Rosen A, Rogers JH, et al. DOT1L as a therapeutic target for the treatment of DNMT3A-mutant acute myeloid leukemia. Blood 2016 Jun 22 [Epub ahead of print].