Epigenetics

Major finding: Combined loss of DNMT3A and TET2 in HSCs promotes lineage-specific transcription factor expression.

Mechanism: DNMT3A and TET2 exhibit cooperative and competitive activity in maintaining DNA methylation.

Impact: Co-occurring *DNMT3A* and *TET2* mutations may synergize to promote transformation by blocking differentiation.

DNMT3A AND TET2 COOPERATE AND COMPETE TO MAINTAIN HSC DNA METHYLATION

Mutations affecting DNA methyltransferase 3A (DNMT3A), which methylates CpG dinucleotides (5mC), and ten-eleven translocation 2 (TET2), which oxidizes 5mC to 5-hydroxymethylcytosine (5hmC), frequently co-occur in lymphoma and leukemia and have similar roles in hematopoietic stem cells (HSC) despite their opposing effects on DNA methylation. To explore the roles of DNMT3A and TET2 in hematologic malignancies, Zhang, Su, Jeong, and colleagues generated single- and double-knockout mice. Bone marrow transplantation revealed that although *Dnmt3a*$^{-/-}$ hematopoietic stem and progenitor cells (HSPC) had similar rates of engraftment as wild-type cells, *Tet2*$^{-/-}$ cells exhibited higher engraftment rates, which were further enhanced by *Dnmt3a* deletion. Further, double-knockout mice developed hematologic disease more rapidly. Genes associated with nucleated red blood cells were upregulated in *Tet2*$^{-/-}$ cells and further upregulated in double-knockout cells, whereas they were downregulated in *Dnmt3a*$^{-/-}$ cells. In contrast, HSC-specific genes were upregulated in *Dnmt3a*$^{-/-}$ cells, downregulated in *Tet2*$^{-/-}$ cells, and further downregulated in double-knockout cells. While loss of *Tet2* or *Dnmt3a* alone preserved the stem-cell program, the combined loss of *Tet2* and *Dnmt3a* promoted lineage-specific regulators, but blocked differentiation. A subset of differentially methylated regions was identified in which *Dnmt3a*$^{-/-}$ cells exhibited reduced methylation, which was further decreased in double-knockout HSCs, indicating that TET2 may synergize with DNMT3A to enhance methylation, whereas other regions exhibited competitive activity. Moreover, the synergistic differentially methylated regions were most enriched for 5hmC, and in double-knockout cells, loss of 5mC and 5hmC were correlated. Loss of 5hmC in the promoter and gene bodies was associated with reduced gene expression in double-knockout cells, whereas 5hmC at the transcription start sites was associated with upregulation. These findings suggest a dual role for TET2 in maintaining HSC gene expression and generating 5hmC that, in cooperation with DNMT3A-deposited 5mC, maintains the repressed state of lineage-specific transcription factors. ■

Zhang X, Su J, Jeong M, Ko M, Huang Y, Park HJ, et al. DNMT3A and TET2 compete and cooperate to repress lineage-specific transcription factors in hematopoietic stem cells. Nat Genet 2016 Jul 18 [Epub ahead of print].

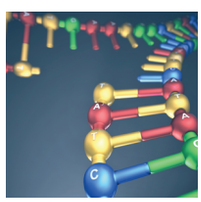
DNA Repair

Major finding: Replication fork protection reduces chemosensitivity in BRCA-deficient cells without restoring HR.

Mechanism: PTIP recruits the MRE11 nuclease to stalled replication forks to promote nascent DNA degradation.

Impact: Drug resistance in BRCA-deficient cells can result from the prevention of nascent DNA degradation.

PTIP LOSS PROTECTS BRCA-DEFICIENT CELLS FROM DNA DAMAGE AND CELL DEATH



BRCA1 and BRCA2 are involved in DNA double-strand break (DSB) repair, and BRCA1/2-deficient cells are hypersensitive to DNA damage due to their reduced ability to repair DSBs by homologous recombination (HR). BRCA-deficient cells are initially sensitive to DNA-damaging agents including cisplatin and PARP inhibitors, but often acquire resistance through HR restoration. As BRCA1 and BRCA2 have DSB-independent functions during replicative stress, Chaudhuri, Callen, and colleagues sought to identify additional mechanisms of DNA damage resistance in BRCA-deficient cells. Inactivation of *Brca1* or *Brca2* in B lymphocytes resulted in replication fork degradation in response to hydroxyurea-induced replication fork stalling. Replication fork degradation is dependent on MRE11 exonuclease activity, and the MLL3/4 complex protein PTIP can inhibit MRE11-dependent DSB resection, thereby preventing BRCA1-dependent HR, but it is not known if PTIP plays a role in replication fork stability. Depletion of PTIP prevented replication fork degradation in response to hydroxyurea. Compared with *Brca1* or *Brca2* depletion alone, double depletion of *Brca1/2* and *Ptip* increased cell viability and reduced chromosomal aberrations, altogether indicating that PTIP can function at stalled replication forks independent of the DSBs. Further, *Ptip* loss promoted replication fork progression and prevented the delayed restart in *Brca2*$^{-/-}$ cells, which was associated with reduced replication fork degradation. PTIP accumulated at stalled replication forks and recruited MRE11. The replication fork stability induced by PTIP loss conferred chemoresistance, likely by enhancing genome stability in response to chemotherapeutics. Data from The Cancer Genome Atlas revealed that in patients with *BRCA2*-mutant tumors treated with platinum-based chemotherapy, high PTIP expression was associated with increased progression-free survival, indicating that PTIP expression might be a potential biomarker for chemoresistance. These findings suggest that in BRCA-deficient tumors, replication fork protection can prevent genome instability and lead to chemoresistance independent of HR restoration. ■

Chaudhuri AR, Callen E, Ding X, Gogola E, Duarte AA, Lee JE, et al. Replication fork stability confers chemoresistance in BRCA-deficient cells. Nature 2016;535:382–7.