Epigenetics

Major finding: CRL4^{VprBP}-mediated monoubiquitylation of TET enzymes promotes their chromatin binding activity.

Concept: VprBP regulation of TET plays an important role in zygotic development and tumor suppression.

Impact: Several oncogenic TET2 mutations in leukemia disrupt its monoubiquitylation or binding to VprBP.

TET MONOUBIQUITYLATION BY CRL4^{VprBP} IS VITAL FOR ITS DNA BINDING

The TET family of methylcytosine dioxygenases (TET1, TET2, and TET3) catalyzes the three-step oxidation of 5-methylcytosine residues in DNA, which repress gene expression, and regulates a number of biological processes via generation of the epigenetic mark 5-hydroxymethylcytosine (5hmC). Deletion of TET enzymes results in developmental defects, and *TET2* is frequently mutated in hematopoietic malignancies, yet little is known about the regulation of TET activity. Nakagawa, Lv, and colleagues found that Vpr-binding protein (VprBP), which acts as a substrate recognition subunit in a complex with DNA-binding protein 1 (DDB1) and cullin 4 (CUL4) (the CRL4^{VprBP} ubiquitin ligase), bound all three TET proteins directly through their C-terminal cysteine-rich dioxygenase domain. VprBP deficiency in fibroblasts reduced 5hmC levels, whereas deletion of *Vprbp* in murine zygotes resulted in decreased paternal DNA hydroxymethylation and defective zygotic development, similar to the phenotype in *Tet3*-deficient zygotes, suggesting that VprBP is vital for TET function. Intriguingly, CRL4^{VprBP} induced the monoubiquitylation of TET proteins; VprBP overexpression enhanced the monoubiquitylation of TET2, whereas depletion of VprBP, DDB1, or CUL4 proteins abolished TET monoubiquitylation, confirming the function of CRL4^{VprBP} as the main ubiquitin ligase for TET proteins. CRL4^{VprBP}-mediated monoubiquitylation occurred at a conserved lysine residue found in all TET proteins (K1299 in TET2). Mutation of the TET monoubiquitylation site did not disrupt the interaction between TET and VprBP, but inhibited TET binding to DNA both *in vitro* and *in vivo* and diminished 5hmC production. Examination of TET2 mutations found in human acute myeloid leukemia revealed several mutations targeting the CRL4^{VprBP} monoubiquitylation site, including K1299 mutations, as well as mutations that disrupted TET2 binding to VprBP. These results demonstrate a role for VprBP in regulating TET DNA binding and activity via monoubiquitylation, and suggest this pathway as a novel mechanism for oncogenic inactivation of TET2. ■

Nakagawa T, Lv L, Nakagawa M, Yu Y, Yu C, D'Alessio AC, et al. CRL4^{VprBP} E3 ligase promotes monoubiquitylation and chromatin binding of TET dioxygenases. Mol Cell 2014 Dec 31 [Epub ahead of print].

Drug Resistance

Major finding: Inhibition of only p110α or p110β is insufficient due to PI3K reactivation by the uninhibited p110 isoform.

Mechanism: PI3K inhibition by isoform-selective inhibitors relieves inhibition of ERBB3 or IGF1R.

Impact: Combined use of PI3Kα and PI3Kβ inhibitors may be required for both *PIK3CA*- and *PTEN*- mutant cancers.

CO-INHIBITION OF p110α AND p110β IS REQUIRED FOR SUSTAINED PI3K SUPPRESSION

Aberrant activation of the phosphatidylinositol 3-kinase (PI3K) pathway by *PIK3CA* mutation, *HER2* amplification, or *PTEN* deletion occurs frequently in solid tumors. Isoform-specific inhibitors of the PI3K catalytic subunits p110α, p110β, and p110δ are in clinical development, but it remains unclear which inhibitors would be most effective in different contexts. In the course of testing breast cancer cell line sensitivity to the p110α inhibitor BYL719, Costa and colleagues found that BYL719 only transiently suppressed PI3K-dependent phosphatidylinositol (3,4,5) triphosphate (PIP_3) production in *HER2*-amplified or *PIK3CA*-mutant cells and AKT phosphorylation in *HER2*-amplified cells. p110β was responsible for the rebound of PIP_3 production and AKT phosphorylation and was reactivated in the presence of BYL719 by the release of p110α-dependent negative feedback regulation of ERBB3 in *HER2*-amplified cells and IGF1R and ERBB3 in *PIK3CA*-mutant cells. Importantly, combined treatment of *HER2*-amplified or *PIK3CA*-mutant breast cancer cells with a combination of p110α- and p110β-selective inhibitors led to greater PI3K pathway suppression than either agent alone and was necessary to induce tumor regression. In the setting of *PTEN*-mutant prostate cancer, which is p110β dependent, Schwartz and colleagues showed that inhibition of p110β with AZD8186 led to relief of feedback inhibition of IGF1R and p110α, which in turn drove PI3K signaling. Co-inhibition of p110α and p110β was required for complete suppression of AKT signaling, but failed to arrest tumor growth *in vivo*. Consistent with the previous finding that reciprocal feedback inhibition exists between the PI3K and androgen receptor (AR) pathways, p110β inhibition activated AR, and only the combination of p110α, p110β, and AR inhibition led to significant tumor regression. These complementary studies highlight adaptive resistance mechanisms that may potentially limit the effectiveness of single-agent p110α or p110β inhibitor therapy and provide support for simultaneously targeting each isoform in PI3K-driven cancers to avoid potential toxicity associated with pan-PI3K inhibition and allow maximal inhibition of each isoform. ■

Costa C, Ebi H, Martini M, Beausoleil SA, Faber AC, Jakubik CT, et al. Measurement of PIP3 levels reveals an unexpected role for p110β in early adaptive responses to p110α-specific inhibitors in luminal breast cancer. Cancer Cell 2015;27:97–108.

Schwartz S, Wongvipat J, Trigwell CB, Hancox U, Carver BS, Rodrik-Outmezguine V, et al. Feedback suppression of PI3Kα signaling in PTEN-mutated tumors is relieved by selective inhibition of PI3Kβ. Cancer Cell 2015;27:109–22.