Commentary

JAK3-mediated phosphorylation of EZH2: a novel mechanism of non-canonical EZH2 activation and oncogenic function

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The enhancer of zeste homologue 2 (EZH2) is the catalytic subunit of polycomb-repressive complex 2 (PRC2) and promotes the trimethylation of the histone H3 lysine 27 (H3K27me3) acting as an epigenetic repressor of gene expression (1). Recent studies have shown that EZH2 is upregulated in various solid and hematologic malignancies and its expression is associated with disease progression and poor prognosis. Numerous studies have demonstrated the presence of EZH2 gain of function mutations in a variety of cancers including B cell lymphomas (2), and activation of upstream oncogenic pathways including RAS, MEK-ERK and Rb-E2F (3) being involved in the activation of EZH2 in cancer cells.

Although the best understood mechanism of EZH2-mediated tumorigenesis is the suppression of gene expression leading to de-differentiation, cell cycle progression, and epithelial-to-mesenchymal transformation, it is known that EZH2 also mediates activation of oncogenic signaling acting independently of PRC2 and promoting cancer progression and resistance to conventional therapies (4). Particularly, it is known that EZH2 interacts with the androgen receptor in prostate cancer cells independently of PRC2, and functions as a transcriptional co-activator inducing the expression of downstream target genes (4). Similarly, EZH2 interacts with RelA/RelB complex co-regulating a subset of NF-kB targets increasing the aggressiveness of breast cancer cells (5).

In a recent publication in *Blood* (6), Yan *et al.* demonstrated that JAK3, a tyrosine kinase activated downstream of cytokine receptors, phosphorylates EZH2 on tyrosine residue 244, altering EZH2 activity and promoting the survival and proliferation of NK/T-cell lymphoma cells. These findings reveal a novel mechanism by which EZH2 promotes tumorigenesis and introduce a new target for the treatment of NK/T-cell lymphoma.

Using co-immunoprecipitation assays, the authors determined that JAK3 and EZH2 interact in NK/T-cell lymphoma cells while inhibition or knockdown of JAK3 leads to increased H3K27me3 trimethylation suggesting that JAK3 might suppresses EZH2 methyltransferase activity. The authors used phosphorylation site prediction programs to determine the EZH2 candidate phosphorylation sites and found that JAK3 can phosphorylate EZH2 on tyrosine residues 22, 244 and 523. Among these sites, only the substitution of EZH2 Y244 was sufficient to inhibit NK-tumor cell growth. Inhibition of JAK3 in NK/T-cell lymphoma cells decreased phosphorylation of EZH2 on tyrosine 244 suggesting that this is a-JAK3 specific phosphorylation site of EZH2. Transfection with mutant EZH2-Y244 led to increased H3K27m3 levels further supporting the conclusion that JAK3-mediated phosphorylation of EZH2 decreases EZH2 trimethylation activity, while promoting NK/T-cell lymphoma cell growth. This novel observation suggests that the oncogenic role of EZH2 in NK/T-cell lymphoma is independent of its epigenetic role as a methyltransferase and part of the PRC2.

To further investigate this hypothesis, the authors performed genomic analysis of NKYS cells transfected with JAK3 siRNA and observed downregulation of EZH2 target genes suggesting increased methyltrasnferase activity of

EZH2 in the setting of JAK3 down-regulation. Interestingly, evaluation of samples from 19 patients with NK/T-cell lymphoma showed strong correlation between JAK-STAT activation gene signature and PRC2-repressed target gene signature further supporting that JAK3 activation is associated with decreased methyltransferase activity thereby de-repressing EZH2 target genes. The investigators induced ectopic expression of EZH2 mutants lacking the methyltransferase activity for H3K27me3 (EZH2 SETΔ), but maintaining the non-canonical transcriptional activation function, and Y244-phosphorylation resistant mutants. EZH2 SETΔ mutant induced increased gene expression compared to EZH2 WT and Y244-phosphorylation resistant mutant suggesting that EZH2 has transcriptional activity independently of its methyltransferase activity and likely induced by phosphorylation in the Y244 residue. ChIP-qPCR analysis revealed EZH2 occupancy in the promoters of non-canonical target genes such as *PMSD1*, *KRAS* and *MAPK15*, which were also positively correlated with JAK-STAT target signature in patient samples. Conversely, JAK3 inhibition decreased the expression of such non-canonical EZH2 target genes but left the expression of canonical EZH2 target genes unaffected. Of note inhibition of EZH2 enzymatic activity by EPZ-6438 did not affect the expression of the non-canonical EZH2 target genes further confirming that EZH2 regulates their expression independently of its methyltransferase activity. Interestingly, these non-canonical genes are involved in oncogenic pathways implicated in proliferation, biosynthesis, stemness, invasiveness, DNA replication and cell cycle progression. This finding supports the conclusion that the non-canonical activity of EZH2 may lead to the acquisition of a more aggressive phenotype in NK/T-cell lymphoma cells.

To explore the mechanism of JAK3-mediated EZH2 activation, the authors performed co-immunoprecipitation assays, which revealed interaction of EZH2 with JAK3 and polymerase II. Phosphorylation of EZH2 on Y244 was critical for this association because a phosphorylation-resistant EZH2 mutant decreased the formation of these complexes and the EZH2 binding on the non-canonical EZH2 target genes promoters but increased the association of EZH2 with components of PRC2. These results demonstrate that phosphorylation of EZH2 by JAK3 on the Y244 increases the interaction with Polymerase II and decreases the association with PRC2 components promoting the expression of non-canonical genes. Interestingly, inhibition of EZH2 catalytic activity and disruption of PRC2 complex did not affect the survival and proliferation of NK/T-cell lymphoma cells despite downregulating H3K27me3. On the contrary JAK3 inhibition by PF956980 decreased cell growth and the percentage of cells in the S phase and increased cell death. These events were abrogated by an EZH2 phospho-mimic mutant suggesting that JAK3 inhibition suppresses the survival and proliferation of NK/T-cell lymphoma cells through inhibition of EZH2 phosphorylation on Y244.

NK/T-cell lymphoma is a rare hematologic malignancy, usually resistant to the conventional chemotherapy due to the expression of P-glycoprotein, which exports anti-tumor agents outside of the tumor cells (7). The 5-year overall survival (OS) rate with chemotherapy followed by radiotherapy does not exceed 50% (8) leading the physicians to choose the option of upfront autologous hematopoietic stem cell transplantation, which leads to improved survival rates ranged from 50–70% (9). These treatment outcomes clearly mandate the development of new therapeutic approaches for this type of lymphoma.

JAK3 is a non-receptor tyrosine kinase associated with activation of STAT signaling and has been related to the development and progression of numerous malignancies. NK/T-cell lymphoma has been found to carry JAK3-activating mutations in about 35.4% of the cases leading to activation of JAK-STAT signaling (10). In their study, Yan *et al*. identified that JAK3 phosphorylates EZH2 in tyrosine residue 244, decreasing EZH2 methyltransferase activity and promoting the transcriptional upregulation of oncogenes such as *PMSD1*, *KRAS* and *MAPK15* involved in cell cycle progression, stemness and DNA replication (6). This novel finding, which connects JAK3 signaling with EZH2 regulation, not only provides better understanding of EZH2 function in NK/T-cell lymphoma but also introduces new targets for novel therapeutic approaches, much needed in this cancer.

Nagel *et al*. showed that EZH2 target genes such as HOXA9 and HOXA10 are up-regulated in NK/T-cell lymphoma suggesting that suppression of EZH2 methyltransferase activity may be involved in the progression of this disease (11). On the contrary, Yan *et al*. showed that EZH2 is upregulated in NK/T-cell lymphoma cells and promotes the expression of cyclin D1, an outcome found to be independent of EZH2 methyltransferase activity (12). Consistent with this finding, Abd Al Kader *et al*. have demonstrated that EZH2 is upregulated (13) and positively correlated with Ki67 labeling index in NK/T-cell lymphomas (14). These conclusions clearly suggest

that EZH2 is involved in the pathogenesis and progression of NK/T-cell lymphoma but not through silencing classical EZH2 target genes via its methyltransferase activity.

EZH2 phosphorylation has been previously associated with progression of solid tumors including prostate cancer (4) and glioblastoma (15). Particularly, AKT1 promotes EZH2 phosphorylation increasing its interaction with androgen receptor leading to increased resistance to androgen deprivation and acquisition of castration resistant phenotype (4). Phosphorylation of EZH2 by cyclin-dependent kinases 1 and 2 at T492 residue disrupts the PRC2 assemblies and decreases EZH2 activity. In contrast, phosphorylation at the T350 and T492 residues by cyclin-dependent kinase 1 decreases methyltransferase activity by inducing EZH2 ubiquitination and degradation (16). Of note, decreased H3K27me3 abundance and methyltransferase activity have been associated with progression of gliomas (17,18) and poor prognosis in ovarian, breast and pancreatic cancer (19). Thus, phosphorylation of EZH2 may lead to decreased methyltransferase activity, which is associated with progression of neoplastic diseases and poor prognosis. These conclusions are consistent with the findings of Yan *et al.*, who determined that JAK3-mediated phosphorylation decreases EZH2 methyltransferase activity but promotes the transcription of oncogenes involved in carcinogenesis in NK/T-cell lymphoma. Most importantly, these results strongly suggest that inhibition of EZH2 in certain malignancies, such as in NK/T-cell lymphoma, will not have therapeutic efficacy. In contrast, in cancers in which diminished EZH2 methyltransferase activity is associated with oncogenesis and disease progression, inhibition of an upstream signaling pathway that suppresses EZH2 activity will be required in order to reverse the pro-survival effects and the enhanced proliferation of cancer cells mediated by diminished EZH2 methyltransferase activation. Particularly, for NK/T-cell lymphoma, JAK3 inhibition appears to be a promising therapeutic approach and evaluation of JAK3 inhibitors as modulators of non-canonical EZH2 activity in clinical trials is warranted.

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Footnote

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