IN THE SPOTLIGHT

An “EZ” Epigenetic Road to Leukemia Stem Cell Metabolic Reprogramming?

Meng Li and Ari M. Melnick

Summary: In this issue of Cancer Discovery, Gu and colleagues developed a mouse model of myeloproliferative neoplasm driven by NrasG12D and Ezh2−/−, which cooperated to induce malignant transformation and metabolic reprogramming of leukemic stem cells at least in part through loss of normal epigenetic regulation of gene expression. Furthermore, their findings point to Ezh1 and branched chain amino acid metabolism as biological dependencies and potential therapeutic targets in myeloid neoplasms.

See related article by Gu et al., p. 1228 (3).

Mutations activating oncogenes and inactivating tumor suppressors can generate aberrant transcriptional and metabolic programming distinct from their normal cell of origin. These reprogramming events may cause cancer cells to become highly dependent on specific molecular pathways for survival and proliferation. In turn, this makes cancer cells vulnerable to specific targeted therapies against these tumor-specific pathways (1). Myeloproliferative neoplasms (MPN) are chronic hematopoietic disorders associated with aberrant expansion of myeloid precursor cells and their differentiated progeny, including polychromatyma vera, essential thrombocytosis, primary myelofibrosis, and prefibrotic primary myelofibrosis. In patients with MPN, JAK2, CALR, and MPL driver mutations are widely observed to activate JAK–STAT signaling to induce constitutive proliferation. However, patients with MPN manifest additional mutant alleles such as those affecting IDH2, TET2, ASXL1, SF3B1, RUNX1, NRAS, and EZH2, independently or concurrently with JAK2, CALR, and MPL mutations. Some of those genetic lesions may contribute to the transformation of MPN into acute myeloid leukemia (AML) and myelodysplastic syndrome, fueled by therapy-resistant leukemia initiating cells/leukemia stem cells (LSC; ref. 2).

In this issue, Gu and colleagues describe a novel form of epigenetic and metabolic reprogramming involved in MPN-to-leukemia transformation caused by NrasG12D and Ezh2 homozygous deletion (3). RAS, a small GTPase, is one of the most frequently mutated oncogenes in cancers. Mutations in RAS, such as G12 mutations, turn it constitutively active in hematopoietic neoplasms (2). EZH2, a histone H3-K27 (H3K27) methyltransferase, is the catalytic active component of polycomb repressive complex 2 (PRC2; ref. 4).

Although gain- and loss-of-function mutations of EZH2 are reported in different types of tumors, those occurring in patients with MPN result in loss of EZH2 function and mostly affect its CXC (cysteine rich) and SET domains, resulting in impaired enzymatic activity (2, 5). Notably, NRAS mutations occur relatively frequently in post-MPN leukemia, and EZH2 mutations are associated with higher risk of leukemic transformation in patients with MPN, suggesting that both mutations may accelerate leukemogenesis. Indeed, Gu and colleagues observed that although mice with NrasG12D/+ and Ezh2−/− alone manifested a modest myeloproliferative phenotype with long latency (median survival >350 days), the two mutations together led to myelodysplasia, severe anemia, leukocytosis, thrombocytosis, and shorter median survival (~70 days). Importantly, NrasG12D+/−; Ezh2−/− mice featured histopathologic features of advanced primary myelofibrosis and 6 of 13 mice progressed into blast-phase disease, which recapitulated leukemic transformation in human MPNs. Hence this model provided a suitable basis to study the process of leukemic transformation and development of LSCs in mice.

EZH2 and PRC2 play critical roles in epigenetic regulation during hematopoiesis. Previous studies showed that derepression of certain Ezh2 target genes could accelerate malignant transformation of myeloid cells (6, 7). Using transcriptomic and epigenetic profiling approaches, Gu and colleagues observed upregulation of genes involved in several metabolic pathways, including branched chain amino acid (BCAA) metabolism, tricarboxylic acid (TCA) cycle, and mTOR signaling. Bcat1, which encodes for the enzyme that catalyzes the first step of BCAA metabolism, was identified as the most highly activated gene in NrasG12D+/−; Ezh2−/− LSCs. Bcat1 expression was normally repressed by PRC2 complex via H3K27me3 but activated by MYC and CEBPA after losing repressive chromatin marks, pointing to a potentially important link between PRC2 and metabolic programming in leukemogenesis. The effects of Ezh2 loss-of-function mutations on epigenetic and metabolic programming could yield epigenetic and metabolic vulnerabilities in LSCs, and hence possible targets for therapies acting through synthetic lethality.

The phenomenon of synthetic lethality refers to the process whereby cancer cells become exceedingly sensitive to
suppression of a molecular mechanism that is dispensable to normal cells, but is lethal to cancer cells (1). Along these lines, EZH1 and EZH2 have partially redundant functions as components of alternative PRC2 complexes (4). Gu and colleagues observed that complete loss of PRC2 function due to deletion of both EZH1 and EZH2 or the essential PRC2 subunit EED results in rapid elimination of hematopoietic stem/progenitor cells (HSPC) from murine bone marrow, suggesting that EZH1 could be a synthetic lethal target in EZH2-mutated cells. Indeed, EZH2−/− LSCs were susceptible to EZH1 knockdown, consistent with the known ability of EZH1 to compensate for EZH2 in EZH2−/− embryonic stem cells (4). Therefore, EZH1 is an epigenetic dependency in EZH2-deficient LSCs. This EZH1 dependency was also observed in other MPN models with EZH2 deletion (6). In contrast to EZH2, loss of EZH1 alone or with NrasG12D/+ failed to yield MPN phenotypes, suggesting that targeting EZH2 would suppress only EZH2-mutant LSCs and would not put normal cells at risk for transformation. It is important to note that gene dosage effects can be crucial to synthetic lethality. Indeed, Gu and colleagues point out that in human myeloid neoplasms, EZH2 mutations are mostly heterozygous, which could make them less EZH1-dependent. Nonetheless, human EZH2-mutant MPN cells manifest marked upregulation of BCAT1, reminiscent of EZH2−/− mice, suggesting that at least certain downstream effects are comparable between the murine and human settings.

LSCs manifest distinct metabolic features as compared with normal HSPCs, such as increased level of oxidative phosphorylation (OXPHOS), relatively low levels of reactive oxygen, sensitivity to disruption of electron transport chain, lack of glucose dependency for energy production, and reliance on amino acids for OXPHOS metabolism (8). BCAAs, including leucine, isoleucine, and valine, are essential amino acids that cannot be de novo synthesized by human cells. Therefore, the activity of the reaction. Dashed arrows indicate inhibited or weak activities.

Figure 1. Different cellular contexts may define the role of EZH2-regulated BCAT1 functions. A, Loss of Ezh2 synergizes with NrasG12D mutation in MPN-to-leukemia transformation to potentiate BCAA synthesis via BCAT1 and trigger downstream mTORC1 signaling (8). B, Loss of Ezh2 can cooperate with Jak2V617F mutation in the polycythemia vera (PV) to primary myelofibrosis (PMF) transition (7). Although BCAT1 expression is upregulated, it might not be as active without the metabolic context of enhanced glutamine metabolism. On the other hand, genes, such as HMGA2, may play more important roles in primary myelofibrosis transition given its role in megakaryopoiesis. C, In AML LSCs, with low EHZ2 and high BCAT1 expression (9), BCAT1 seems to favor BCAA degradation and depletion of αKG, which lead to DNA hypermethylation and stabilization of HIF1α via impaired activity of TET2 and EGLN1, respectively. The orange boxes highlight mutational and gene-expression context; proteins in red letters are possible therapeutic targets in each context, and metabolites are in blue. Level or activity changes are indicated by arrows on the right (up, increase; down, decrease). Symbols in gray color indicate possible regulations that were not studied in publications (3, 7). The solid black arrows show the directions of metabolic reactions and thickness shows the activity of the reaction. Dashed arrows indicate inhibited or weak activities.
data point to BCAT1-mediated BCAA metabolism as a key metabolic vulnerability in NrasG12D−/−;Ezh2−/− LSCs.

EZH2 depletion was shown to promote MPN progression in cooperation with other alleles such as Jak2V617F and Tet2−/− (6, 7), although these other studies did not investigate BCAT1 induction. So, does BCAT1 induction occur in all MPNs/AMLs regardless of Ezh2-knockout cells expression is consistently increased in Ezh2-knockout cells with Jak2V617F background. They also observed that CD34+ HSPCs from patients with AML featured decreased H3K27me3 at the BCAT1 promoter compared with normal HSCs. Consistently, human AML cells with low expression of EZH2 had higher expression of BCAT1. Thus, BCAT1 may be commonly required in the transformation of MPN to AML LSCs. However, to fully address this point it is important to carefully consider cellular context, where EZH2 and BCAT1 may play different roles. One critical reason why BCAT1 is required in the NrasG12D−/− model is because NrasG12D−/− strongly enhanced uptake of glutamine to support the BCAT1 transamination reaction of BCAA synthesis. On the other hand, it is not clear whether BCAT1 is required to the synergy between Ezh2−/− and Jak2V617F, which promoted polycythemia vera to primary myelofibrosis transition via Ezh2 target genes, such as HMG2A (7). BCAT1 might not be as important in this context perhaps if Jak2V617F does not enhance glutamine uptake as much as NrasG12D−/− (Fig. 1B). In the context of AML, BCAT1 was also shown to be required for LSC functions (9), but in this case BCAT1 was needed to promote BCAA degradation and Glu synthesis, as opposed to the case of NrasG12D−/−;Ezh2−/− MPNs (Fig. 1C). This BCAA metabolic activity caused AML LSCs to exhaust α-ketoglutarate (αKG), resulting in increased DNA methylation and HIF1α stabilization even in the absence of TET2 and IDH mutations (9). Therefore, although BCAA metabolism may be a metabolic vulnerability in both MPNs and AMLs, the mechanism through which BCAT1 mediates these effects may be highly dependent on mutational and cellular metabolic contexts.

These considerations raise several questions that could be addressed with further research. First, what is the impact of glutaminolysis and TCA cycle metabolism in mTORC1 signaling and LSC metabolism? Leu and Gln can potentially trigger mTORC1 activation via glutaminolysis (10). NrasG12D−/−;Ezh2−/− LSCs manifest active glutamine usage and increased expression of TCA cycle genes, which may contribute to mTORC1 signaling in disease progression and favor LSC’s metabolic dependency on oxidative phosphorylation. Thus, increased TCA cycle activity may consume excessive intracellular αKG to facilitate DNA hypermethylation and HIF1α activity. Second, what is the source of BCKA? Diets containing high Leu significantly accelerated cancer progression in NrasG12D−/− mice. NrasG12D−/−;Ezh2−/− LSCs need extracellular BCKA to synthesize BCAAs, but BCKAs can only be obtained from food or protein degradation, so that understanding the source of BCKA may help to control the level of Leu in cancer cells. Dietary limitation of BCAA intake might thus be beneficial for patients with MPN and EZH2 mutations.

Disclosure of Potential Conflicts of Interest

A.M. Melnick reports receiving a commercial research grant from Janssen and is a consultant/advisory board member for Epizyme, Constellation, and KDAC Pharma. No potential conflicts of interest were disclosed by the other author.

Published first September 3, 2019.

REFERENCES

An "EZ" Epigenetic Road to Leukemia Stem Cell Metabolic Reprogramming?

Meng Li and Ari M. Melnick