# The Role of TET2 in Hematologic Neoplasms

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*TET2* encodes an enzyme that hydroxylates methylcytosine and is frequently targeted by loss-of-function mutations in myelodysplasia, myeloproliferative disorders, and acute myeloid leukemia. In this issue of *Cancer Cell*, two studies show that inactivation of *Tet2* enhances hematopoietic stem cell self renewal and promotes the development of myeloproliferative disorders.

*TET2* (tet oncogene family member 2, or Ten-Eleven Translocation 2) is a member of a family of genes with important roles in epigenetic programming, embryonic stem cell maintenance, and early development (Ito et al., 2010). A potential role in the pathogenesis of hematologic neoplasms was first recognized with the identification of rearrangements of *TET1* in acute leukemia (Lorsbach et al., 2003). Recently, a plethora of reports have identified a high frequency of loss-of-function mutations of the *TET2* gene in hematologic malignancies, including myelodysplastic syndrome, myeloproliferative neoplasms (MPN), and de novo and secondary acute myeloid leukemia (AML). Notably, up to 50% of chronic myelomonocytic leukemia (CMML), a mixed myelodysplastic and myeloproliferative condition with a propensity to progress to AML, harbor *TET2* mutations. Moreover, CMML with *TET2* alterations appear to harbor fewer concomitant copy number alterations and mutations compared to *TET2* wild-type CMML (Abdel-Wahab et al., 2009; Jankowska et al., 2009). Consequently, there is intense interest in elucidating the role of TET2 in hematopoiesis, epigenetic regulation, and leukemogenesis.

A key role of the TET family of enzymes is to convert 5-methyl-cytosine (mC) to 5-hydroxymethyl-cytosine (hmC) (Figure 1) (Tahiliani et al., 2009). The role of 5hmC is incompletely understood; however, recent genome-wide analyses in mouse embryonic stem cells have shown hmC to be enriched in actively transcribed genes and in the promoter regions of Polycomb-repressed developmental regulators (Wu et al., 2011). The *TET2* mutations in hematologic tumors occur throughout the gene and include missense, nonsense, and frameshift mutations. Missense mutations are commonly found in the functional domains of TET2, including the cysteine-rich and catalytic double-stranded β helix domains, suggesting that the mutations result in loss of TET2 function. *TET2* mutations are associated with low hmC levels and global hypomethylation (Ko et al., 2010). Moreover, mutations of *TET2* and the isocitrate dehydrogenase genes *IDH1/IHD2* are mutually exclusive in AML, and the metabolic product of the neomorphic IDH1/2 mutant proteins, 2-hydroxyglutarate, inhibits the catalytic activity of TET2 (Figure 1) (Figueroa et al., 2010; Xu et al., 2011). *TET2* mutations may be acquired prior to *JAK2* mutations in MPNs, but also at the progression of MPN to AML. The *Tet* genes are expressed in early hematopoietic progenitors, with downregulation during differentiation, and inhibition of *Tet2* expression promotes expansion of monocyte-macrophage lineage cells in vitro. Together, these observations suggest that TET2 has key roles in epigenetic regulation and hematopoiesis, and that *TET2* mutations are central events in leukemogenesis. However, these hypotheses have not been formally tested in vivo.

Two studies in this issue of *Cancer Cell* report murine models of *Tet2* inactivation that establish important roles for Tet2 in hematopoiesis and the development of myeloproliferative disease, and show that lymphoid as well as myeloid malignancies harbor *TET2* mutations. Initial in vitro studies by Moran-Crusio et al. (2011) showed that short hairpin RNA-mediated silencing of *Tet2* in murine bone marrow cells resulted in enhanced replating ability in colony forming assays, upregulation of the hematopoietic stem, and progenitor cell marker c-Kit and a decrease in global hmC levels. These authors engineered a mouse model with conditional *Tet2* loss in the hematopoietic compartment by targeting exon 3, a commonly mutated exon in human myeloid malignancies (Moran-Crusio et al., 2011). Quivoron et al. (2011) created two different *Tet2* deficient mouse models, where one had an insertion of a β-galactosidase-neomycin cassette in the *Tet2* gene ($Tet2^{LacZ}$), giving rise to a dysfunctional Tet2-β-galactosidase transcript. The second was a conditional knockout of *Tet2* that, upon Cre-mediated recombination, resulted in expression of a truncated Tet2 protein lacking the last 490 amino acids of the carboxy terminus, including the catalytic domain (Quivoron et al., 2011).

In the study of Moran-Crusio et al. (2011), *Vav-cre*+$Tet2^{-/-}$ mice lacked *Tet2* expression in the hematopoietic lineage, and like shRNA-silenced *Tet2* cells, *Vav-cre*+$Tet2^{-/-}$ deficient cells exhibited serial replating. The $Tet2^{-/-}$ cells had upregulated levels of c-Kit and myeloid progenitor markers (e.g., CD34 and FcγR), and a gene expression profile similar to that of common myeloid progenitors, indicating that these cells are c-$Kit^{+}$ myeloid progenitors. *Tet2* deficiency was shown to result in progressive perturbations in hematopoiesis in vivo in both studies. *Tet2* loss resulted in expansion of the hematopoietic stem cell (HSC) compartment and granulocyte-macrophage progenitor population in the marrow, splenomegaly, and extramedullary hematopoiesis. Quivoron et al. (2011) also observed the expansion of an aberrant immature (CD19+B220low) lymphoid population in *Tet2* deficient mice. With age, two out of the three mouse models ($Tet2^{LacZ}$ and *Vav-cre*+$Tet2^{-/-}$) developed a disease reminiscent of human CMML that was

characterized by myeloproliferation and splenomegaly.

*TET2* alterations in patients are most commonly heterozygous, suggesting that TET2 haploinsufficiency may be sufficient to result in hematopoietic progenitor defects and myeloproliferation. Accordingly, in vitro and in vivo experiments using $Tet^{+/-}$ mice demonstrated that the heterozygous cells behaved in a similar fashion as *Tet2* null cells, with serial replating potential and expansion of myeloid lineage cells. $Tet^{+/-}$ cells also exhibited a competitive advantage over *Tet2* wild-type cells in transplantation assays, although unsurprisingly this expansion occurred over a longer time period than with $Tet2^{-/-}$ cells.

The role of *TET2* alterations in lymphoid malignancies has been less well studied. Several patients with *TET2* mutant myeloid malignancies have been noted to develop lymphoma, prompting Quivoron et al. (2011) to sequence *TET2* in a range of mostly mature B and T cell neoplasms. This identified deleterious mutations in 2% of B cell and approximately 12% of T cell lymphomas, notably in 30% of angioimmunoblastic T cell lymphoma, an aggressive neoplasm of CD4+ T-lymphocytes. Prior *TET2* genotyping studies in myeloid disorders have shown that the *TET2* mutations are commonly present in hematopoietic progenitors, and interestingly, the same phenomenon was observed in several patients with lymphoma. One patient that harbored two *TET2* mutations subsequently developed AML, and both mutations were also detected in the AML sample. A second patient with T cell lymphoma also had two *TET2* mutations, one of which was also detected in a fraction of HSCs, indicating that these alterations are commonly acquired in early hematopoietic progenitors, irrespective of the lineage of the subsequent neoplasm. Further studies were performed to determine if the *TET2* mutations identified in lymphomas were derived from early hematopoietic progenitors and identified the mutations in progenitor cells with myeloid differentiation potential.

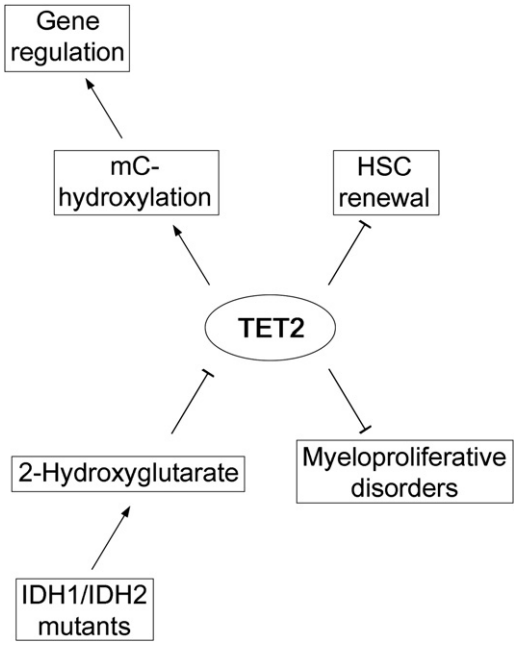


**Figure 1. Schematic of the Regulatory Network of TET2**
HSC, hematopoietic stem cell; mC, 5-methyl-cytosine. 2-Hydroxyglutarate is the metabolic product of mutated, neomorphic IDH1 and IDH2 proteins.

Together, these data provide important mechanistic insights into the role of TET2 in normal and malignant hematopoiesis. These studies show that Tet2 loss results in expansion of hematopoietic stem and progenitor cell populations and directly contributes to myeloproliferation, consistent with a central role of *TET2* disruption by deletion or sequence mutation in the pathogenesis of lymphoid as well as myeloid disorders (Figure 1). Moreover, the mutations in both lineages of malignancy are commonly acquired in early hematopoietic progenitors of multilineage potential, indicating that enhanced self renewal consequent on TET2 inactivation is an important mechanism of transformation. Moreover, genomic profiling and sequencing efforts are implicating an increasing number of genes with roles in cytosine and histone modification in a range of hematologic and solid tumors including *IDH1, IDH2, DNM3TA,* and the polycomb repressive complex 2 genes *EZH2* and *SETD2* and others, indicating that perturbations in epigenetic programming are key events in tumorgenesis and that these epigenetic alterations may be driven by genetic alterations in the genes encoding this machinery. Future work modeling these pathways in tumorigenesis will be of interest, and the models described here provide an important platform to study these genes and to test the efficacy of therapeutic agents designed to modulate these epigenetic alterations.

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