

Genetic and non-genetic mechanisms of inflammation may promote transformation in leukemia

Paresh Vyas

MRC Molecular Haematology Unit, Oxford Biomedical Research Center Hematology Theme, Oxford Centre for Haematology, Weatherall Institute of Molecular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK

Non-genetic mechanisms of transformation are still relatively understudied and poorly understood. In this issue of Cell Stem Cell, Muto et al. provide preclinical evidence that loss of TRAF6 expression promotes transformation through a MYC-dependent mechanism that may be modulated by environmental inflammatory signals.

Study of hematopoietic stem/progenitor cell biology, and the perturbations leading to clonal dominance, and eventual transformation to acute leukemia, has often opened new fields that have had general implications. For example, the finding that somatic mosaicism commonly leads to clonal expansion of human blood stem cells, known as clonal hemopoiesis (CH) (reviewed in Jaiswal and Ebert, 2019), opened our eyes to the fact that this is happening in nearly all tissues, and is likely to impact the health of those tissues. Importantly, CH is more common as humans age and is associated with a 12-fold increased risk of developing blood cancer, particularly acute myeloid leukemia (AML). Unsurprisingly, many of the single-nucleotide variants and small insertions and deletions that mark CH clones are in genes that are recurrently mutated in AML, and they often initiate mutations that give rise to functional pre-leukemic stem cells (Jan et al., 2012; Shlush et al., 2014). How these recurrent mutations, and associated epigenetic changes, result in clonal dominance is still poorly understood and the subject of very active research. What is clear is that the pre-leukemic stem progenitor cells require additional events to transform them to the leukemic phenotype, and this has resonance across many cancers.

Through extensive sequencing, an atlas of recurrent genetic mutations has been identified in AML and other cancers. This has provided a platform for functional validation and dissection of the mechanisms of cooperation between initiating and transforming genetic mutations (Labuhn et al., 2019). However, what has been less well studied are non-genetic mechanisms that cooperate with initiating genetic events to transform clonally selected somatic stem cells. In this issue of Cell Stem Cell, Muto et al. (2022) provide an interesting example of such a non-genetic mechanism. Starting with a loss-of-function screen in murine Tet2 null hematopoietic stem progenitor cells (HSPCs) (loss-of-function mutations in TET2 are common in human CH), the authors showed that decreased expression of TNF-receptor-associated factor 6 (TRAF6) was a common hit. TRAF6 is an intracellular protein best known for modulating complex signaling pathways downstream of the TNF receptor super family. One of TRAF6's activities is to function as a E3 ubiquitin protein ligase. In a key follow-up genetic experiment, the authors crossed Tet2f/f mice with Traf6f/f mice and showed that transplantation of mice with bone marrow cells harboring the conditional deletion of both genes resulted in an aggressive, transplantable, myeloproliferative phenotype with leukemic features (myeloproliferative neoplasm/acute myeloid leukemia, or MPN/AML). This clearly showed the cooperativity of loss of Tet2 and Traf6 in causing transformation. Through an elegant series of biochemical experiments, the authors then demonstrated that loss of TRAF6 in Tet2/ cells results in increased expression of genes regulated by MYC and

that this in part, or whole, may be due to decreased TRAF6- dependent ubiquitination of MYC on residue K143, leading to increased acetylation of MYC on the same amino acid residue. Importantly, decreased ubiquitination of MYC did not change MYC protein stability and MYC mutants that mimicked K143-acetylated MYC increased expression of MYC target genes. Finally, expression of an acetylation-defective MYC mutant in a human leukemic cell line led to less aggressive leukemia in an immunodeficient mouse model. Taken together, these data support the model whereby loss of TRAF6 expression can transform TET2 deficient pre-leukemic stem/progenitor cells by modulating MYC function (Figure 1).

So, what do we know about TRAF6 and human AML? Monoallelic loss of chromosome 11p13, which includes the TRAF6 gene, occurs in 3% of AML patients. TRAF6 variants are observed in 1% of AML cases, but it is unclear if these are functionally relevant. Using publicly available RNA sequencing datasets, the authors show that TRAF6 mRNA expression is reduced in AML cells, especially in patients with an antecedent pre-leukemic condition, compared to normal cells. However, the correlation between low TRAF6 expression and AML is difficult to interpret as mRNA expression is often influenced by cell differentiation state, and this will vary between AML patients and between bulk normal cells compared to bulk AML cells. Nevertheless, AML patients with lower mRNA TRAF6 expression, compared to patients with higher TRAF6 expression, have a modestly, but significantly, poorer survival and higher leukemic burden. Lower expression of TRAF6 mRNA in AML cells was correlated with higher TRAF6 promoter methylation but the mechanism of reduced TRAF6 mRNA expression is unclear. So, Muto et al. (2022) provide some clues that suggest that reduced TRAF6 expression is a cooperative event in leukemic transformation. More importantly, this work provides a solid foundation for additional work in human CH, MPN, and AML samples.

Finally, this work raises potentially interesting connections between inflammation and cancer initiation and progression. CH is associated with excess cardiovascular mortality and coronary atherosclerosis (Genovese et al., 2014; Jaiswal et al., 2014; Jaiswal et al., 2017). Animal studies show that mature Tet2/ myeloid cells are pro-inflammatory and contribute to plaque formation (Fuster et al., 2017; Jaiswal et al., 2017). Conversely, in inflammatory atherosclerosis, blood stem cell proliferation is increased, with a consequent increase in acquisition of somatic variants and somatic evolution (Heyde et al., 2021). Thus, CH and somatic evolution both feed inflammation and are fed by inflammation. In this regard, it would be interesting to establish and are fed by inflammation. In this regard, it would be interesting to establish the relationships between an inflammatory environment, level of TRAF6 expression, and MYC function, in normal and CH HSPCs and the rates of expansion and clonal evolution of these cells. Understanding the links between different genetic germline and somatic states, and inflammation, in regulating clonal expansion and somatic evolution is an important field where many discoveries are awaiting to be made. These discoveries will be vital in understanding the links between inflammation, aging, and cancer. We need to unpick the mechanistic sequence of events in this process to eventually enable therapeutic targeting of pre-leukemic cells (and potentially other pre-cancerous cells) before they are fully transformed.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

- Fuster, J.J., MacLauchlan, S., Zuriaga, M.A., Polackal, M.N., Ostriker, A.C., Chakraborty, R., Wu, C.L., Sano, S., Muralidharan, S., Rius, C., et al. (2017). Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science* 355, 842–847. <https://doi.org/10.1126/science.aag1381>.
- Genovese, G., Kähler, A.K., Handsaker, R.E., Lindberg, J., Rose, S.A., Bakhoum, S.F., Chambert, K., Mick, E., Neale, B.M., Fromer, M., et al. (2014). Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N. Engl. J. Med.* 371, 2477–2487. <https://doi.org/10.1056/NEJMoa1409405>.
- Heyde, A., Rohde, D., McAlpine, C.S., Zhang, S., Hoyer, F.F., Gerold, J.M., Cheek, D., Iwamoto, Y., Schloss, M.J., Vandoorne, K., et al. (2021). Increased stem cell proliferation in atherosclerosis accelerates clonal hematopoiesis. *Cell* 184, 1348–1361 e1322. <https://doi.org/10.1016/j.cell.2021.01.049>.
- Jaiswal, S., and Ebert, B.L. (2019). Clonal hematopoiesis in human aging and disease. *Science* 366, eaan4673. <https://doi.org/10.1126/science.aan4673>.
- Jaiswal, S., Fontanillas, P., Flannick, J., Manning, A., Grauman, P.V., Mar, B.G., Lindsley, R.C., Mermel, C.H., Burt, N., Chavez, A., et al. (2014). Age-related clonal hematopoiesis associated with adverse outcomes. *N. Engl. J. Med.* 371, 2488–2498. <https://doi.org/10.1056/NEJMoa1408617>.
- Jaiswal, S., Natarajan, P., Silver, A.J., Gibson, C.J., Bick, A.G., Shvartz, E., McConkey, M., Gupta, N., Gabriel, S., Ardissino, D., et al. (2017). Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. *N. Engl. J. Med.* 377, 111–121. <https://doi.org/10.1056/NEJMoa1701719>.
- Jan, M., Snyder, T.M., Corces-Zimmerman, M.R., Vyas, P., Weissman, I.L., Quake, S.R., and Majeti, R. (2012). Clonal evolution of Mature myeloid cells Late progenitor cells HSC Early progenitor cells AML Preleukemic mutations Transforming Events Pre-leukemia TRAF6+ Ubiquitination of Myc K143 Acetylation of Myc K143 Myc function Role of inflammation ? Figure 1. Cooperation between low TRAF6 protein levels and oncogenic transformation of pre-leukemic cells
- Left, hierarchy of normal hemopoietic differentiation as hemopoietic stem cells (HSCs) differentiate into mature myeloid blood cells. Acquisition of somatic pre-leukemic genetic events, such as mutation of Tet2, leads to a pre-leukemic phenotype. Transformation into acute myeloid leukemia (AML) could occur by non-genetic events that lead to decreased expression of TRAF6 and consequent changes in MYC ubiquitination and acetylation at residue lysine 143 (K143) that increase transcriptional activity of MYC. The role of inflammation in this process, particularly in reducing TRAF6 expression, remains to be elucidated. preleukemic hematopoietic stem cells precede human acute myeloid leukemia. *Sci. Transl. Med.* 4, 149ra118. <https://doi.org/10.1126/scitranslmed.3004315>.
- Labuhn, M., Perkins, K., Matzk, S., Varghese, L., Garnett, C., Papaemmanuil, E., Metzner, M., Kennedy, A., Amstislavskiy, V., Risch, T., et al. (2019). Mechanisms of Progression of Myeloid Preleukemia to Transformed Myeloid Leukemia in Children with Down Syndrome. *Cancer Cell* 36, 123–138 e110. <https://doi.org/10.1016/j.ccell.2019.06.007>.
- Muto, T., Guillaumot, M., Yeung, J., Fang, J., Bennett, J., Nadorp, B., Lasry, A., Redondo, L.Z., Choi, K., Gong, Y., et al. (2022). TRAF6 functions as a tumor suppressor in myeloid malignancies by directly targeting MYC oncogenic activity. *Cell Stem Cell* 29, 298–314.

Shlush, L.I., Zandi, S., Mitchell, A., Chen, W.C., Brandwein, J.M., Gupta, V., Kennedy, J.A., Schimmer, A.D., Schuh, A.C., Yee, K.W., et al.; HALT Pan-Leukemia Gene Panel Consortium (2014). Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. *Nature* 506, 328–333. <https://doi.org/10.1038/nature13038>.