A sheep in wolf's clothing? Wild-type P53 disguises as mutant to promote leukemogenesis

Margaret A. Ferris[1] and John S. Welch[2]
[1]Department of Pediatrics, Washington University and [2]Department of Internal Medicine, Washington University, St Louis, MO, USA
E-mail: jwelch@wustl.edu
https://doi.org/10.3324/haematol.2022.280671

In this issue of Haematologica, Tuval and colleagues[1] describe misfolded P53 (pseudo-mutant P53) as a marker of preleukemic cells in patients with *DNMT3A*-mutated acute myeloid leukemia (AML). *TP53* is mutated across human cancers, including AML. P53 functions to protect genomic stability; interestingly, this can be achieved by activating distinct pro- and anti-apoptotic pathways. To add to this complexity, multiple *TP53* isoforms can be expressed and post-translational modifications further influence P53 function within specific cellular contexts.[2] Thirty years ago, Gannon and colleagues identified a monoclonal antibody (PAb240) that distinguished mutant P53 from wild-type, and theorized that this antibody recognized an epitope that was protected in the wild-type conformation.[3] Zheng and colleagues later found a series of *TP53* wild-type AML cell lines that express P53 in the mutant conformation, referred to as "pseudo-mutant P53".[4] Since then, the pseudo-mutant conformation has been observed in alternative splice forms of wild-type P53, and these can decrease MDM2 binding, preventing degradation of activated P53.[5] The current study expands on these findings, comparing expression of this pseudo-mutant conformation in different leukemia subpopulations in patients without *TP53* mutations.

AML is a clonal disease associated with subclonal heterogeneity within individual patient samples. Two separate models of leukemic hierarchy have emerged. Mutation analysis suggests the presence of a founding clone, subsequent cooperating mutations in subclones,[6] and a related preleukemic state, clonal hematopoiesis (CH), associated with clonal mutations but retained normal hematopoietic maturation.[7] Second, immunophenotypic analysis separates hematopoietic stem cells from progenitors and from more mature cells. Recent studies have sought to harmonize these two models, demonstrating that within a patient, immunophenotypic preleukemic-hematopoietic stem/progenitor cells (preL-HSPC) can be identified with driver mutations (*DNMT3A*) but not cooperating mutations found in the AML blasts (e.g., *NPM1c*); in contrast to leukemic blasts, these preL-HSPC retain their capacity for multi-lineage differentiation. Xenograft experiments show that these preL-HSPC have a growth advantage over non-mutated HSPC.[8] Somatic *TP53* mutations have been found at the preL-HSPC stage in ~20% of cases, conferring a selective advantage in xenograft models.[9]

The current study examines P53 protein conformations in preL-HSPC from AML patients. Importantly, the use of AML cases with *DNMT3A* and *NPM1* mutations again allowed for separation of leukemic blasts (both mutations present) from preL-HSPC (*DNMT3A*-mutated, *NPM1* WT). The authors then assessed P53 for pseudo-mutant conformation. Similar to the findings in AML cell lines,[4] Tuval and colleagues found expression of the pseudo-mutant P53 in *TP53* wild-type primary AML patient samples. Using mass cytometry, the samples were further separated into leukemic blasts (immunophenotype defined at diagnosis) and preL-HSPC (CD34+CD33-CD15-CD11b-CD19-CD79b-CD3-CD16-CD45RA-), and the ratio of pseudo-mutant to wild-type conformation P53 (PM/WT-CR) was examined in each individual cell. Interestingly, heterogeneity in P53 conformations was identified: within blasts the wild-type conformation was dominant (PM/WT-CR = 0.53), but in the less abundant preL-HSPC, the pseudo-mutant confirmation was enriched (PM/WT-CR = 3.06) (Figure 1). The high PM/WT-CR appears specific to the preL-HSPC and was not observed in normal stem cells (cord blood PM/WT-CR = 1.22) or cells from a patient with *DNMT3A*-mutated clonal hematopoiesis (*DNMT3A*^{R882H}-CH PM/WT-CR = 0.53) (Figure 2).

The authors go on to use a xenotransplant model to determine whether the high PM/WT-CR leads to a selective

Figure 1. In this issue Tuval and colleagues demonstrate that wild-type *TP53* may take on folding patterns similar to mutant *TP53* (pseudo-mutant), and this may contribute to leukemic transformation.

growth advantage of the preL-HSPC. The variability of engraftment potential of patient-derived xenografts limited the power of these studies as only one of the nine AML samples lead to engraftment of preL-HSPC (#160005); the other samples engrafted the immunodeficient mouse marrow with leukemic blasts or non-leukemic stem cells (*DNMT3A* WT). Tumor #160005 had one of the more modest PM/WT-CR (~1.3). However, treatment with a P53-stabilizing peptide, pCAP-250, decreased the engraftment potential of the preL-HSPC, but not of the non-preL-HSPC, suggesting that the balance of wild-type and pseudo-mutant P53 contributed to the engraftment and expansion capacity of the preL-HSPC (Figure 3). Single cell RNA sequencing analysis of engrafted cells showed pCAP-250 treatment was associated with a reduction in specific subsets of cells. However, mass cytometric analysis of the engrafted cells did not correlate PM/WT-CR level on susceptibility to pCAP-250, suggesting heterogeneity of pCAP-250 effects or pseudo-mutant P53 dependency that remain uncharacterized (Figures 5 and 6). Therefore, P53 may contribute to early transformational programs through both mutant and non-mutant effects. *TP53* missense mutations are present in clonal hematopoiesis patients,[10] are expressed at the preL-HSPC stage, and are associated with expressed proteins that alter sensitivity to chemotherapy and early transformation.[9] Likewise, during transformation of induced pluripotent stem cells, overexpression of the Δ133p53α isoform inhibits wild-type P53-inducible cellular senescence pathways, augmenting the reprogramming capacity.[5] Now Tuval and colleagues demonstrate the heterogenous presence of pseudo-mutant P53 protein in *TP53* wild-type hematopoietic cells, and the potential of pseudo-mutant P53 to influence transformation potential in preL-HSPC, distinguishing them from cells of clonal hematopoiesis where pseudo-mutant P53 does not appear dominant or active.

These provocative findings are thus far limited to small sample sizes and within *DNMT3A*-mutant/*NPM1*-wild-type-defined preL-HSPC, which were cleverly chosen to distinguish preL-HSPCS from leukemic blasts. Additional cases and types of mutations in preL-HSPCs will need to be examined to further define the frequency, distribution, and phenotypes of pseudo-mutant P53 in hematopoietic transformation. Knowing that P53 can have anti- and pro-apoptotic effects and that non-R882 *DNMT3A* mutation-driven CH is less likely to progress to AML, it will be interesting to determine the distribution and phenotypes of pseudo-mutant P53 in additional forms of preL-HSCP and CH. The mechanisms enabling pseudo-mutant folding in hematopoietic cells also remain uncharacterized, although splice variation and posttranslational modifications have influenced P53 folding patterns in other cellular contexts. This study serves as a starting point for defining the role of wild-type P53 modifications in leukemogenesis, and suggests that in the right context, wild-type P53 might put on wolf clothing to unexpectedly contribute to leukemic transformation.

Disclosures

No conflicts of interest to disclose.

Contributions

MAF and JSW wrote the manuscript.

Funding

JSW receives research funding from Janssen Pharmaceuticals and from Notable Labs.

References

1. Tuval A, Brilon Y, Azogy H, et al. Pseudo-mutant P53 as a targetable pheneotype of DNMT3A-mutated pre-leukemia. Haematologica. 2022;107(11):2548-2561.
2. Anbarasan T, Bourdon JC. The emerging landscape of p53 isoforms in physiology, cancer and degenerative diseases. Int J Mol Sci. 2019;20(24):6257.
3. Gannon JV, Greaves R, Iggo R, Lane DP. Activating mutations in p53 produce a common conformational effect. A monoclonal antibody specific for the mutant form. EMBO J. 1990;9(5):1595-1602.
4. Zheng A, Castren K, Säily M, Savolainen ER, Koistinen P, Vähäkangas K. p53 status of newly established acute myeloid leukaemia cell lines. Br J Cancer. 1999;79(3-4):407-415.
5. Fujita K. p53 isoforms in cellular senescence- and ageing-associated biological and physiological functions. Int J Mol Sci. 2019;20(23):6023.
6. Welch JS, Ley TJ, Link DC, et al. The origin and evolution of mutations in acute myeloid leukemia. Cell. 2012;150(2):264-278.
7. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371(26):2488-2498.
8. Shlush LI, Zandi S, Mitchell A, et al. Identification of pre-leukaemic haematopoietic stem cells in acute leukaemia. Nature. 2014;506(7488):328-333.
9. Lal R, Lind K, Heitzer E, et al. Somatic TP53 mutations characterize preleukemic stem cells in acute myeloid leukemia. Blood. 2017;129(18):2587-2591.
10. Chen S, Liu Y. p53 involvement in clonal hematopoiesis of indeterminate potential. Curr Opin Hematol. 2019;26(4):235-240.