COMMENTARY

Insights from TARGET-seq: Inflammation drives *TP53*-mediated clonal evolution

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Approximately 10%–20% myeloproliferative neoplasms (MPNs) can progress into blast-phase MPNs, also termed secondary acute myeloid leukemias (sAMLs), presenting dismal prognoses. sAML exhibits a distinctive clinical and pathological profile compared to *de novo* AML, marked by a higher incidence of erythroid leukemias and reduced responsive to conventional chemotherapies, resulting in a median survival of approximately 6 months. *TP53* alterations are prevalent adverse events in leukemic transformation (LT), occurring in around one-third of sAML subjects. Notably, *TP53* mutations persist at a low level for years in chronic-phase MPN, but during LT, the mutational burden rapidly accumulates through a multi-hit mechanism, involving secondary mutations, loss of heterozygosity, copy-number variations, and parallel evolution within distinct clonal populations. Despite these insights, the precise mechanisms governing interactions between nongenetic factors and pre-existing genetic variants, initiating malignant transformation, remain unclear.

Rodriguez-Meira et al.[1] conducted TARGET-seq analysis on hematopoietic stem and progenitor cells (HSPCs) derived from 14 sAML subjects with *TP53* mutations, 9 age-matched healthy donors, and 8 patients with myelofibrosis, providing valuable insights into the intratumoral heterogeneity of HPSCs and various clonal evolution patterns during LT. Their findings revealed three main HPSC clusters in sAML: (1) a *TP53* multi-hit cluster with erythroid bias, possibly correlating with a reduced *CEBPA/GATA1* expression ratio; (2) a cluster of *TP53* multi-hit leukemic stem cells, defined by a 44-gene "p53LSC signature," offering precise predictive value for survival; and (3) a *TP53*-WT (wild-type) pre-leukemic cluster characterized by quiescence signatures, differentiation defects, and elevated expression of specific cell-extrinsic inflammatory mediators. Upon transferring the *TP53*-WT cell cluster to extended *ex vivo* culture, a tendency for cells to recover differentiation capabilities was observed, indicating that these functional and molecular abnormalities are likely cell-extrinsically mediated. Additionally, analysis of chronic-phase MPN HSPCs from individuals who either did or did not develop *TP53*-mutated sAML (pre-*TP53* sAML) revealed that *TP53* homozygous cells from pre-*TP53* sAML displayed an elevated inflammatory profile compared to their heterozygous counterparts. To further investigate the impact of chronic inflammatory stimuli on *TP53* clonal evolution, the researchers conducted competitive transplant experiments in murine models subjected to inflammatory stimuli. They observed $Trp53^{R172H/+}$ HSPC enrichment and a reduction in WT competitors, highlighting the fitness advantage conferred by *TP53* mutations to cells suffering inflammation-induced DNA damage, ultimately resulting in clonal expansion (Figure 1B).

The research of Rodriguez-Meira et al. emphasized how single-cell multi-omics techniques can be applied to address intratumoral heterogeneity in disease biology. MPN serves as an excellent model for studying intratumoral cell heterogeneity and clonal evolution; thus, it is a particularly tractable cancer evolution model. In recent years, advancements in single-cell genomics techniques applied to MPN have offered unprecedented insights, answering many scientific questions within the MPN field. Indeed, classic single-cell sequencing technologies, primarily single-cell RNA sequencing (scRNA-seq), have unveiled transcriptomic signatures and chromosomal alterations in cancer cells, providing valuable insights for understanding disease mechanisms and cell-cell interactions. However, they often lack the resolution to detect genomic alterations comprehensively, hindering the correlation of scRNA-seq data with mutation analysis owing to limited genomic coverage. TARGET-seq is emerging as a promising tool, enabling simultaneous profiling of genomic mutations and unbiased whole transcriptomes in individual cells. It combines this with cell surface antigen profiling using index flow cytometry, providing major advantages over uniomic single-cell sequencing technologies (Figure 1A). In this context, the application of TARGET-seq by Rodriguez-Meira et al. played a pivotal role in uncovering the transcriptional characteristics of HSPC subclones with varying *TP53*-mutant statuses, deciphering complex clonal hierarchies, and addressing substantial interpatient variability.

Despite the advantages, TARGET-seq has limitations, particularly in discovering novel mutations relevant to disease progression. This is because it requires prior knowledge of pre-identified mutations. For diseases with ambiguous pathogenic mutations, a comprehensive approach may involve an initial phase of bulk whole-genome/-exome sequencing for identifying suspicious mutations, followed by subsequent TARGET-seq analysis of the candidates.

In addition to TARGET-seq, other approaches exist for acquiring single-cell multi-omics data, facilitating clone tracing at the single-cell level. For example, genotyping of transcriptomes (GoT) allows targeted detection of specific mutations of interest in combination with the transcriptome of the same cell.[2] Similar to TARGET-seq, GoT can only detect a limited number of genes with pre-identified mutations using custom-designed primers. In comparison, GoT has higher throughput owing to highly efficient droplet microfluidics for single-cell isolation, whereas TARGET-seq offers improved coverage and accuracy in mutation detection through simultaneous amplification of both genomic and transcriptomic regions. Additionally, mitochondrial DNA (mtDNA) mutation analysis combined with scRNA-seq may be a powerful tool for clone tracking, given that mtDNA mutation rates are much higher than nuclear DNA mutation rates, allowing mtDNA to be an innate barcode for inferring clonal relationships.[3]

Advancements in sequencing technology now enable the clonal evolution and phenotypic traits of mutations to be revealed at the single-cell level, offering profound insights into the interplay between inflammation and malignant progression. These findings align with previous studies in the clonal evolution field. For example, Cai et al. found that *Tet-2*-knockout HSPCs exhibit heightened activity in the interleukin-6 (IL-6)/Shp2/Stat3/Morrbid pathway, promoting clonal expansion, especially under inflammatory stress.[4] Although these studies revealed the role of inflammation in enhancing the fitness and survival of pre-leukemic clones, they did not unravel the dynamic changes occurring simultaneously at the genotypic, transcriptomic, and phenotypic levels at single-cell resolution, owing to the constraints of low-resolution technologies for assessing mutation statuses. Conversely, the research of Rodriguez-Meira et al. represents a substantial progression in comprehending the intricate interplay between chronic inflammation and the amplification of clonal evolution, particularly in cases with multi-hit *TP53* mutations. Notably, unlike murine studies, this study involved direct analysis of human specimens from clinical cohorts representing crucial stages of disease progression. Thus, the role of inflammation in causing DNA damage, driving genetic evolution, and conferring a fitness advantage to p53 mutant cells is now supported by evidence from both murine and human studies.

Nevertheless, the findings of Rodriguez-Meira et al. raise numerous questions to be resolved in future investigations. First, inflammation typically involves the activation of inflammatory cells and the release of various cytokines in response to external agents or internal injuries. However, chronic inflammation in MPN, as mentioned in the paper, is narrowly defined by an increase in specific proinflammatory cytokines, including interferon γ. Second, although it reported

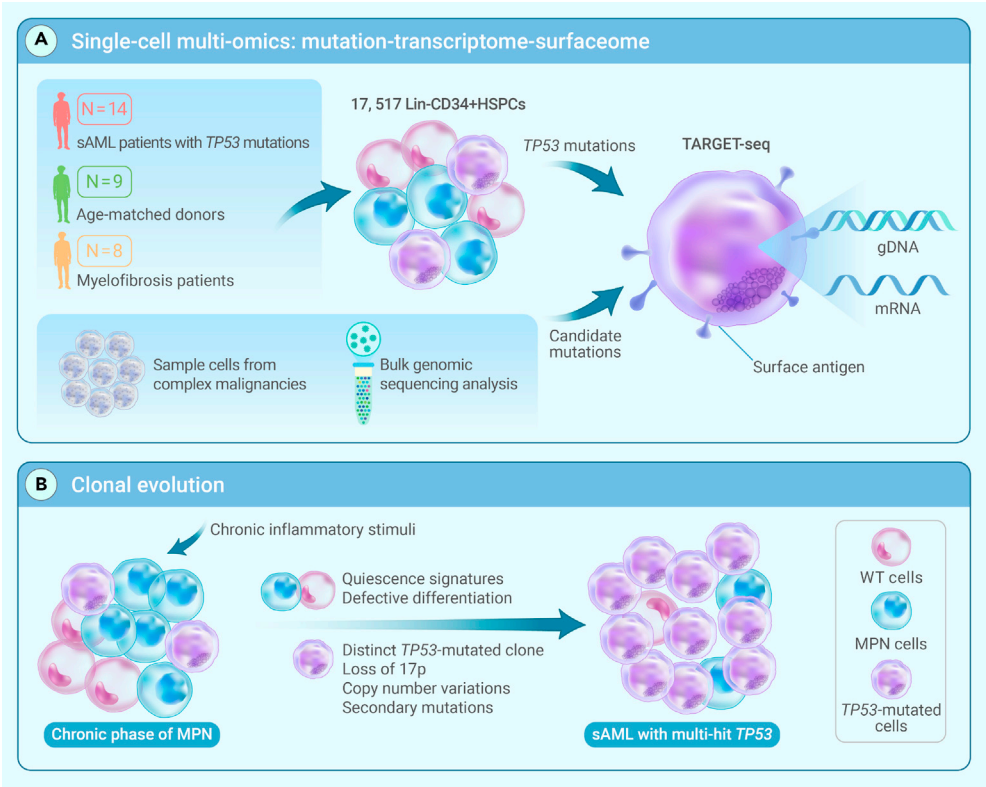

Figure 1. Revealing genetic evolution in HSPCs during *TP53*-mutation-driven leukemic transformation through TARGET-seq (A) TARGET-seq detects targeted mutations within individual cells, covering both genomic and transcriptomic mutations. In this study, this method was combined with surface antigen profiling to distinguish different cells. In cases with unknown mutations, TARGET-seq requires initial whole-genome/-exome sequencing and subsequent TARGET-seq analysis of candidates. This approach facilitates the exploration of mechanisms underlying disease onset and progression at both multi-omics and single-cell levels. (B) Exposure to chronic inflammatory stimuli results in the accumulation of multi-hit *TP53* mutations. Concurrently, wild-type HSPCs exhibit quiescence signatures and impaired differentiation, cooperatively promoting the progression to sAML.

inflammatory signatures characterized by elevated expression levels of oxidative phosphorylation, DNA repair, and interferon response genes in *TP53*-heterozygous HSPCs, as well as more pronounced effects in *TP53*-homozygous cells from the same patients with pre-*TP53*-sAML, the key inflammatory signals or pathways driving *TP53*-mutant leukemic evolution remain unidentified. Further experiments are required to elucidate the critical inflammatory mediators involved. Targeting these mediators may help inhibit the inflammation and potentially delay malignant evolution.

Many malignancies harboring *TP53* mutations, especially for AML, are associated with dismal outcomes. Despite extensive efforts using novel agents to restore functional structures, prevent WT protein degradation, and explore immunotherapies, *TP53* mutations persist as a formidable challenge. Inflammation as a therapeutic target warrants further investigation, necessitating a precise understanding of critical inflammatory mediators and underlying molecular mechanisms to identify specific targets. Notably, *TP53*-mediated LT represents just one route of early-stage clonal evolution. Clonal hematopoiesis (CH) with preleukemic mutations, including *DNMT3A*, *TET2*, *ASXL1*, *SRSF2*, *TP53*, *IDH2*, *SF3B1*, and *JAK2*, has garnered substantial attention. Prior studies have revealed elevated CH rates in patients with solid tumors, and CH has been linked to disruptions in inflammatory signaling in various contexts. Therefore, the role of inflammation in clonal evolution may lead to the development of innovative strategies for earlier cancer intervention and the establishment of a new treatment paradigm.

In conclusion, the study by Rodriguez-Meira et al. exemplifies the broad applicability of TARGET-seq analysis and also indicates a wide application future of single-cell multi-omics techniques, including proteome, methylome, interactome, RNA modifications, chromosome accessibility, and topography, hopefully enhancing our comprehension of malignant cell behaviors.[5] Given the prevalence of *TP53* mutations across various cancers and their importance in leukemogenesis, this research provides valuable insights into intratumoral heterogeneity and its evolution during disease progression, offering a potential blueprint for investigating similar complexities in other cancers. Additionally, it lays a foundation for considering early interventions targeting inflammation to mitigate rapid clonal evolution and malignant transformation in cancers driven by *TP53* mutations.

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DECLARATION OF INTERESTS

The authors declare no competing interests.