ToTAL1y degraded - rapid dTAG proteolysis of TAL1 in T-cell acute lymphoblastic leukemia

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In the current issue of Haematologica, Ong and colleagues interrogate the regulatory mechanisms of TAL1 in T-cell acute lymphoblastic leukemia (T-ALL) cells by taking advantage of the very powerful dTAG degradation system.[1,2] TAL1, together with GATA3 and LMO2, are among a select group of master transcription factors (TF) that orchestrate normal thymocyte development.[3] When such TF are dysregulated through chromosomal translocation or somatic mutation, thymocytes undergo differentiation arrest, often, but not always, at a developmental time point where these genes are not normally expressed. *TAL1*, for instance, is usually downregulated after the double-positive stage of thymic development. In T-ALL, chromosomal lesions, such as the ~80 kb deletion that juxtaposes the ubiquitously active *STIL* promoter to *TAL1*,[4] or the *TAL1* neo-enhancer mutations that create binding sites for MYB,[5] lead to its continuous overexpression during thymic development and differentiation arrest at the post-cortical stage. Class-defining lesions, such as those affecting *TAL1*, *TLX1*, *TLX3* and *MEF2C*, define their gene expression program and are typically not seen together in the same T-ALL. Ong and colleagues offer a credible rationale for why this is the case.

TAL1 relies on secondary hits in signaling pathways to lead to full transformation to T-ALL. For instance, in mouse models of Tal1-induced T-ALL, three-quarters of tumors harbored activating-mutations in *Notch1*,[6] suggesting cooperativity between these two factors. However, the molecular crosstalk between NOTCH1 and TAL1 has not been fully elucidated.

Up to now, studies interrogating the transcriptional program induced by TF oncogenes in T-ALL have mainly used small-interference RNA (siRNA) or short-hairpin RNA (shRNA) knockdown approaches.[5,7] Despite their validity, the knockdown achieved by these tools is often slow and incomplete, prone to off-target effects, and relies on electroporation or viral transduction that can induce indirect gene expression changes. Instead, Ong and colleagues introduced a mutant FKBP12 sequence in-frame to the 3' of TAL1 using CRISPR/Cas9. On the addition of dTAG, a heterobifunctional small molecule capable of binding to both mutant FKBP12 and the E3 ligase system, TAL1 is rapidly degraded within 2 hours, giving the opportunity to analyze the kinetics and dynamics of direct TAL1 target genes with minimal cell manipulation.

By employing acute protein degradation of TAL1 for a period of 2-72 hours in Jurkat T-ALL cells, the authors classified three subsets of TAL1 target genes: i) group A genes that show the most rapid and dynamic downregulation on TAL1 depletion, i.e., are positively regulated by TAL1, ii) group B genes that exhibit less extreme downregulation and whose expression reaches a plateau, and iii) group C genes that are upregulated on TAL1 depletion i.e., negatively regulated by TAL1. Whereas genes belonging to group A comprise the oncogenic TAL1 signature and are likely to be direct TAL1 targets, genes that belong to group B were partially dependent on TAL1, and group C genes, most likely represent indirect negative targets. The latter include genes such as *RAG1*, *RAG2* and *PTCRA*, which are known to be positively regulated by E-proteins, highlighting the antagonism that can occur with TAL1 at specific loci.[7]

Utilizing ATAC sequencing, chromatin immunoprecipitation (ChIP) sequencing and H3K27ac high-throughput ChIP (HiChIP), the authors further characterized the chromatin features of each subgroup, thus inferring the regulatory mechanisms of TAL1. By taking as an example the *ALDH1A2* and *SIX6* gene loci, group A genes that show a lack of chromatin accessibility in hematopoietic stem cells or normal T cells, chromatin loops at enhancers were lost upon TAL1 degradation. Importantly, the role of TAL1 in chromatin loop formation has only been addressed in erythroid cells where TAL1 mediates the interaction between the γ-globin gene and its enhancer through LDB1.[8] The authors then explored whether TAL1 or LMO1 alone, or together, were sufficient to establish an open chromatin state in TAL1-negative

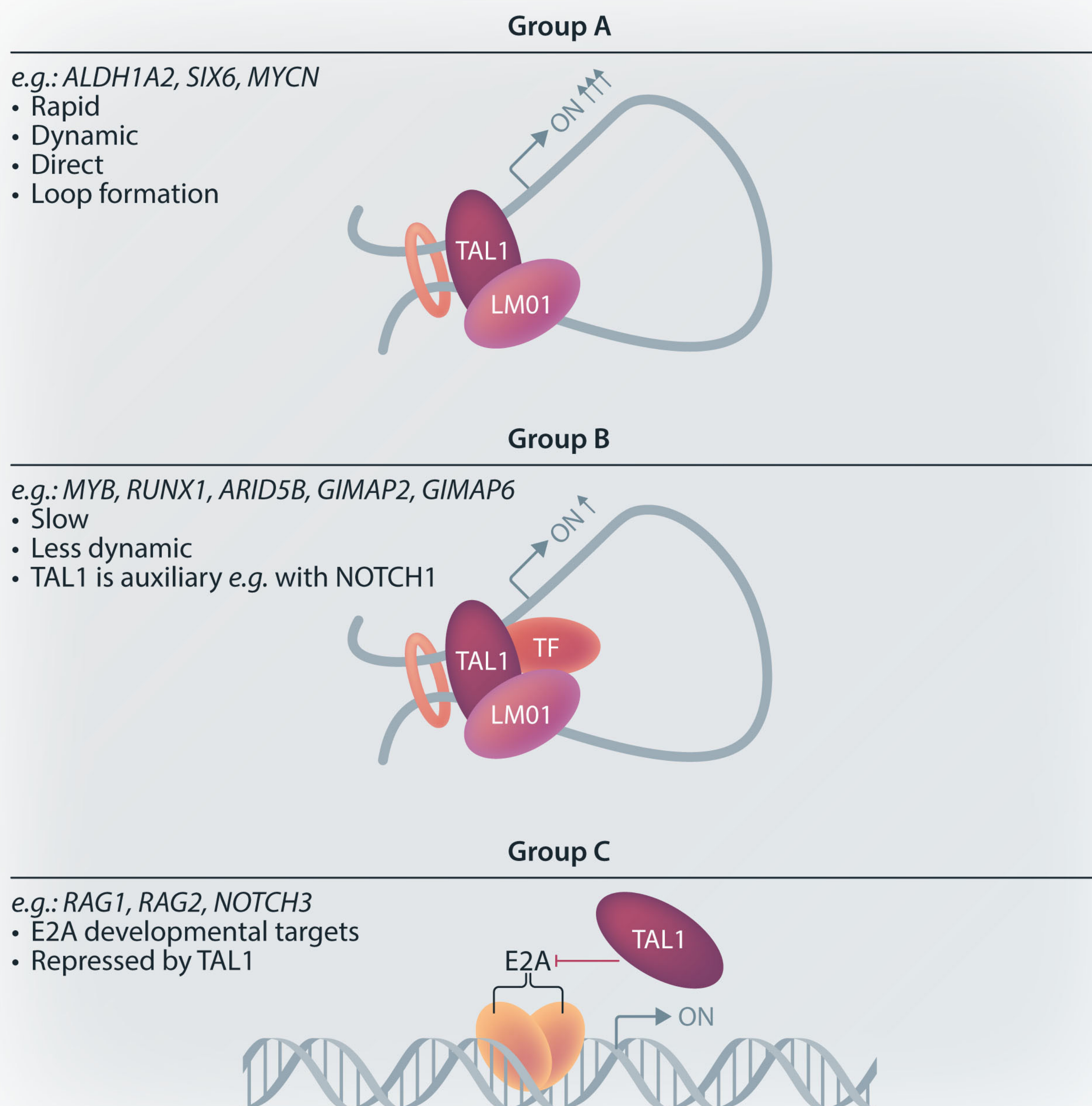

Figure 1. Schematic representation of TAL1 target genes as demonstrated by dTAG-induced TAL1 depletion. Target genes are classified into group A, group B and group C genes. Group A genes are directly dependent on TAL1 and regulated via chromatin looping. Group B genes are partially dependent on TAL1 and require additional transcription factors. Group C genes are indirectly repressed by TAL1 via inhibition of E2A activity. TF: transcription factor.

HBP-ALL cells. Only when TAL1 and LMO1 were expressed together was there significant upregulation of target genes, highlighting the cooperativity between these two factors, with relevance to the observation that T-ALL patients often have activating lesions in both TAL1 and LMO1/2.[9] A subset of these genes were direct TAL1 target genes identified by the dTAG approach, including *ALDH1A2* and *SIX6* gene, which exhibited gain of chromatin accessibility by ATAC sequencing and a dramatic increase of mRNA expression. In contrast, group B genes, that include members of the previously recognized transcriptional regulatory circuit, such as MYB and RUNX1, were characterized by dense chromatin interactions with only marginal reductions in chromatin loops upon TAL1 depletion. This finding most likely reflects a more intricate pattern of regulation of group B genes, where in addition to TAL1, other factors are likely to play a role. Focusing on the *GIMAP* locus, a known TAL1 and NOTCH1 target,[10] a reduction of chromatin loops was achieved by the combination of TAL1 degradation and NOTCH1 inhibition. These findings indicate that one regulatory factor can compensate for the loss of another, but the loss of both has a severe effect.

Lastly, the authors tested the functionality of TAL1 in HBP-ALL cells, a TAL1-negative cell line of the TLX3 subgroup. Combined overexpression of TAL1 and LMO1 resulted in cell proliferation arrest concomitant with downregulation of NOTCH1 target genes, including *MYC*. Overexpression of MYC partially rescued cells from proliferative arrest. Although a previous study where TLX3 was overexpressed in TAL1-positive cell lines did not interrogate cell proliferation[11] the results

indicate that TF oncogenes that define the molecular T-ALL subtypes may have antagonistic effects, thus explaining the fact that *TLX1/3* translocations and *TAL1* activating lesions are mutually exclusive in T-ALL. These findings underscore the cell context-dependent nature of TAL1 in T-ALL, acting cooperatively with NOTCH1 in one cell type and inhibiting the NOTCH1 downstream program in another.

Overall, the study from Ong and colleagues provides a comprehensive view of the TAL1-induced transcriptional program and addresses context-dependent roles of TAL1 that can further provide a rationale for improved targeted therapies. Importantly, this is the first study where TAL1-mediated chromatin looping is recognized as a mechanism that sustains gene activation in T-ALL.

Disclosures

No conflicts of interest to disclose.

Contributions

Both authors contributed equally.

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