Chemical restriction of PU.1 genomic binding sites activates alternate gene networks

Blockade of primary genomic binding sites with small molecules causes redistribution of the transcription factor PU.1 to alternative binding sites; its transcriptional activity at these sites activates secondary gene networks that drive myeloid cell differentiation.

This is a summary of:
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The mission

Transcription factor (TF) localization governs cell fate by dictating which genomic material is utilized by the cell. Furthermore, in diseases such as cancer, TF localization is often corrupted. However, the mechanisms that control TF localization are not well understood, and our ability to target TFs with small molecules to treat diseases and direct cell fate is inadequate. Thus, we set out to determine whether chemicals could interfere with key DNA–TF interactions in cells, and whether this inference could be applied to redirect cell fate. To address this issue, we focused on the hematopoietic TF PU.1, which directs a well-characterized transcriptional program governing cell fate and is central in myeloid leukemia development[1,2].

The discovery

We used small-molecule heterocyclic diamidines (DB2115, DB2373 and DB2826), which bind to DNA sequences containing AT-rich PU.1 binding sites and are known to inhibit PU.1–DNA interactions[3,4], to study the genome-wide consequences of blocking this particular binding site. Through extensive genomic localization studies using cleavage under targets and tagmentation (CUT&Tag), we discovered that PU.1 was rapidly displaced from AT-rich PU.1 binding sites throughout the genome as a direct result of heterocyclic diamidine treatment in both leukemia cell lines (MOLM13, THP1, HL60 and MV411) and primary leukemia cells. However, an unexpected consequence of this inhibition was that PU.1 was subsequently redistributed to GC-rich PU.1 binding sites, which are reported to be lower affinity sites for PU.1[5] (Fig. 1a). This PU.1 redistribution cascaded into a reorganization of the accessible chromatin (as judged by assay for transposase-accessible chromatin (ATAC-seq)) and the production of an alternate PU.1-driven transcriptome (as assessed by RNA sequencing). Time-course analysis revealed that the redistribution of PU.1 to GC-rich binding sites occurred prior to the opening of the chromatin at these same sites, supporting the view that PU.1 is a non-classical pioneer transcription factor (that is, a factor that can access 'locked away', nucleosome-bound DNA)[6].

The ultimate consequence of this PU.1 redistribution was a rewiring of the downstream gene network, causing decreases in the expression of stem cell signature genes and increases in the expression of myeloid cell identity genes. This rewiring allowed acute myeloid leukemia (AML) cells to overcome their pathological differentiation block and differentiate down the myeloid lineage (Fig. 1b). Thus, these small-molecule heterocyclic diamidines represent a class of 'TF redistributors' that can rewire TF networks to alter cell fate.

Future directions

The pharmacological restriction of PU.1 binding sites in this study has revealed the potential to redistribute TFs such as PU.1 between sites, and uncovers the hierarchy of PU.1 binding preferences under physiological conditions. TF redistributors such as DB2115, DB2373 and DB2826 provide a tunable approach to study the fast biology of PU.1 and might allow us to unravel the complex relationships between chromatin components that dictate gene expression in cells. Furthermore, the potential of TF redistributors as therapeutics is highly attractive and theoretically plausible, particularly in instances in which PU.1 is central to disease development. Of course, the effects of TF redistributors on healthy hematopoietic cells also needs to be assessed.

Although PU.1 is, to our knowledge, the only member of the ETS family of TFs that is sensitive to chemical displacement, further investigations should explore whether other TFs are affected by binding site restriction. This exploration could help to determine whether heterocyclic diamidine-sensitive co-factors are contributing to the displacement of PU.1 from binding sites, and whether these small molecules could be used to study other TFs.

The discovery of pharmacologically mediated TF redistribution opens up new routes of investigation and we are excited to develop more potent versions of these compounds, determine the physiological mechanisms of PU.1 redirection, and evaluate the therapeutic potential of PU.1 redistribution in preclinical models of disease, including AML and myelodysplastic syndromes.

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EXPERT OPINION

"This paper explores a tool compound that can block binding of the transcription factor PU.1. I was initially dubious about the specificity of the compound, but the authors provided substantial compelling data to convince me that this compound does work and that its mechanism of action is as predicted. The findings are novel and will be a conceptual advance." **Jason Carroll, University of Cambridge, Cambridge, UK.**

FIGURE

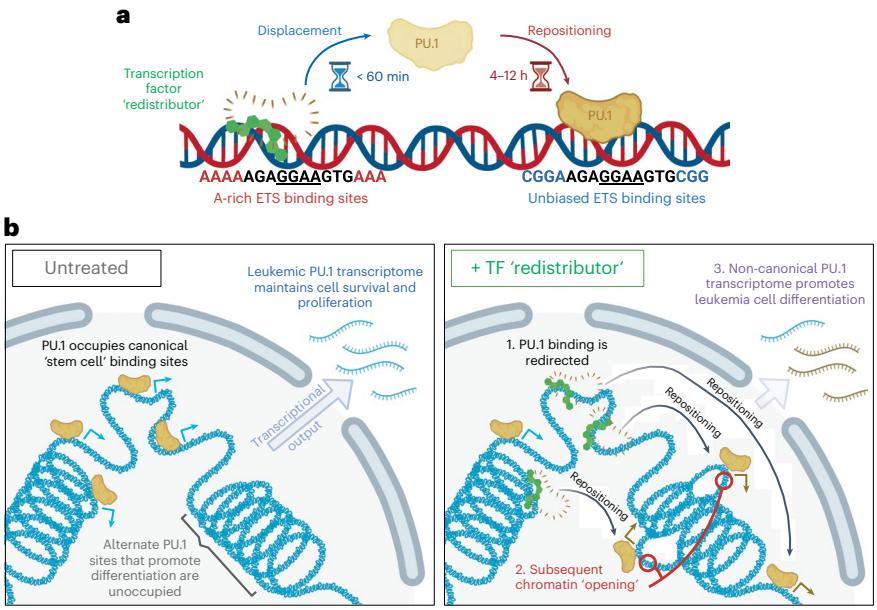

Fig. 1 | Molecular and cellular consequences of pharmacological PU.1 redistribution. a, Transcription factor redistributors displace PU.1 from AT-rich PU.1 binding sites, which leads to redistribution to secondary GC-rich PU.1 binding sites. **b**, In leukemia cells, genomic AT-rich PU.1 binding sites contribute to the downstream production of a leukemia-supportive transcriptional program. Administration of TF redistributors, namely DB2115, DB2372 and DB2826 in this study, redirects PU.1 binding and activity, subsequently opening new chromatin regions and driving the production of an alternate PU.1-driven transcriptome, which facilitates myeloid lineage differentiation. Panels **a** and **b** created with BioRender.com.

BEHIND THE PAPER

Our first experiment for this project was highly puzzling. We had examined the genomic localization of PU.1 following treatment with our binding site 'inhibitors'; however, we did not find the expected global reduction in PU.1 binding. After ruminating on this unexpected observation and partaking in many discussions within our collaborative team, we realized that we were not accounting for a key fundamental attribute of our compounds, that is, that they prefer AT-rich PU.1 binding sites. After re-examining the PU.1 localization data for GC content we had our 'eureka' moment. We had not appreciated that PU.1 could rapidly find alternate binding sites to occupy after displacement from its canonical AT-rich binding sites. From here, all of the other pieces of the puzzle started to fall into place, and turned this project into a very exciting and satisfying investigation. **S.J.T. & U.S.**

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FROM THE EDITOR

"The authors show that chemically driven blockade of PU.1 binding sites leads to its genome-wide redistribution, and that this PU.1 network rewiring causes human acute myeloid leukemia cells to differentiate. It will be interesting to test whether this strategy will be efficacious in vivo, particularly in a clinical context. Additionally, future studies should investigate if this type of approach can be easily extended to regulate other transcription factors." **Tiago Faial, Chief Editor, *Nature Genetics*.**