Transcriptional regulation of cell fate plasticity in hematopoiesis

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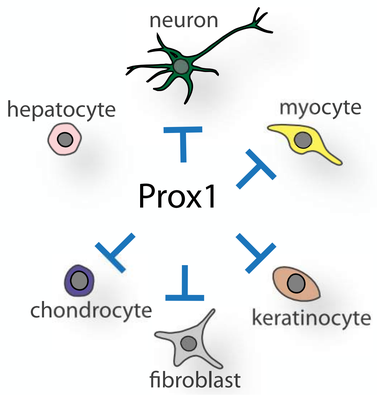
Repressing Alternate Fate



During differentiation, activating the correct lineage is not enough. Competing fates must also be actively repressed to ensure stable and irreversible commitment.

Pioneer transcription factors initiate lineage programs by binding closed chromatin and increasing accessibility. However, this same accessibility can inadvertently expose elements of alternative fates.

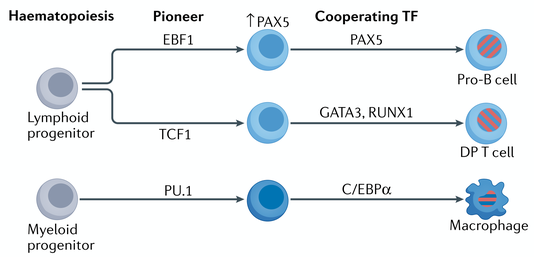
Safeguard repressors counterbalance this risk by silencing non-selected trajectories and restricting plasticity during critical transitions.

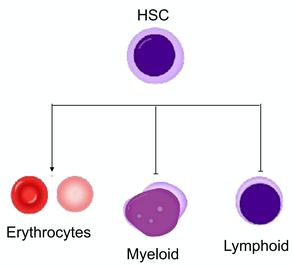
We previously identified PROX1 as a safeguard repressor in hepatocyte differentiation, where it represses alternative gene programs.

In this study, we investigate whether similar repressors operate in hematopoiesis to reinforce lineage fidelity under dynamic regulatory cues.

Lineage Commitment in Blood

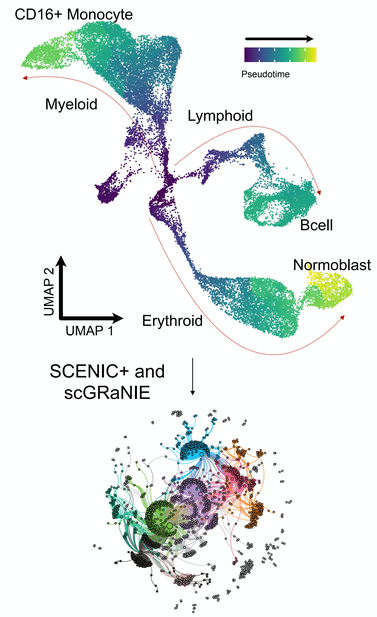
PU.1, EBF1, and TCF1 act early to bias hematopoietic progenitors toward distinct fates, influencing downstream regulators such as PAX5, RUNX1, and C/EBPα. These transcription factors establish lineage competence and help partition chromatin accessibility among competing programs.





Despite strong activation cues, hematopoietic progenitors retain the potential to adopt alternative identities. Without repression of competing programs, fate instability and aberrant differentiation can occur. We hypothesize that active repression mechanisms restrict plasticity during early branching events, especially between myeloid and erythroid trajectories.

Inferring GRNs from Single-Cell Data



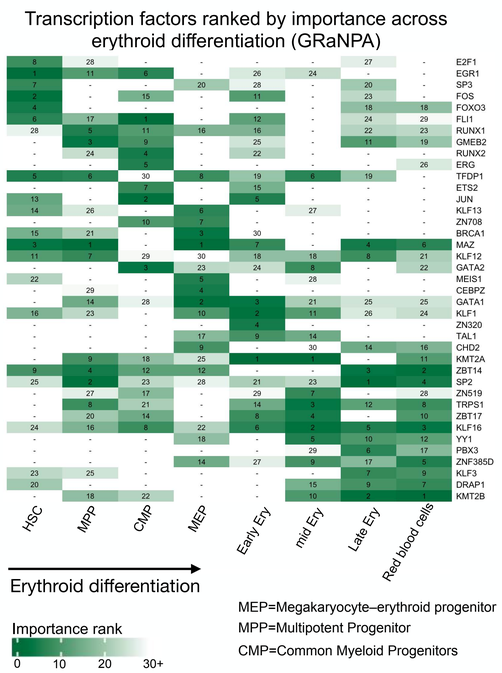
We used a publicly available multiome bone marrow dataset (Multiome; RNA + ATAC) to reconstruct differentiation trajectories from hematopoietic stem cells toward erythroid, myeloid, and lymphoid fates.

Pseudotime was inferred from UMAP space, and lineage-specific pseudobulk profiles were constructed to reflect fate progression.

From these profiles, we inferred gene regulatory networks using two complementary tools: **scGRaNIE**, which integrates chromatin accessibility and expression, and **SCENIC+**, which infers regulons from co-expression and motif analysis.

These GRNs were evaluated using **GRaNPA** to prioritize transcription factors involved in lineage commitment and potential repression of alternate fates.

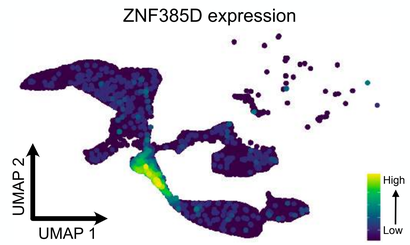
Identifying Key Regulators



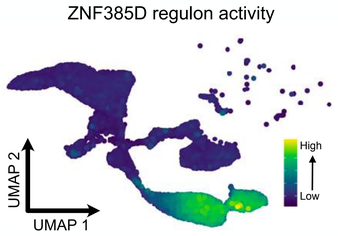
MEP=Megakaryocyte–erythroid progenitor
MPP=Multipotent Progenitor
CMP=Common Myeloid Progenitors

We applied GRaNPA to rank transcription factors across stages of erythroid differentiation. As expected, GATA2 and TAL1 ranked highly in early stages, while GATA1 dominated the intermediate phase. In addition, several less-characterized factors emerged at later stages.

ZNF385D was one such factor: although its expression is largely restricted to early erythroid progenitors and HSCs, it ranked highly in late erythroid stages. The UMAP on the right shows its regulon activity is absent in mature cells, suggesting that its loss of activity may be functionally important. These findings highlight ZNF385D as a candidate repressor whose silencing could help consolidate erythroid fate.

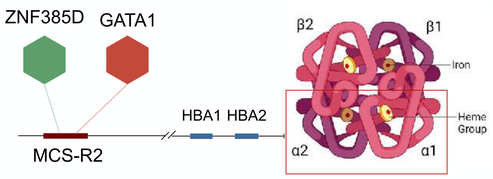


ZNF385D & α-globin



ZNF385D regulon activity is highest in early and progenitor stages but declines sharply in terminal erythroid cells, suggesting a repressive role during differentiation. Based on target gene analysis, ZNF385D appears to regulate several erythrocyte-associated genes, including those at the alpha-globin locus. Its deactivation may be necessary to permit full activation of erythroid programs in late stages, pointing to a potential function as a safeguard that delays premature commitment.

One such target is the alpha-globin super-enhancer MCS-R2, a major regulatory element that drives HBA1 and HBA2 expression. While GATA1 is a known activator of this enhancer in late erythropoiesis, the early binding pattern of ZNF385D suggests it may act on the same element to restrict premature expression during earlier stages.



Discussion

ZNF385D emerges as a promising transcriptional regulator in late erythropoiesis based on its GRaNPA rank and regulon activity.

Although its role in hematopoiesis is not well characterized, predicted repression of erythroid-specific targets suggests a function in stabilizing lineage commitment.

Binding near the MCS-R2 enhancer, which regulates the alpha-globin genes, further supports a role in controlling terminal erythroid maturation.

We hypothesize that ZNF385D limits unwanted transcriptional programs during differentiation and functions as a safeguard repressor.

Ongoing CRISPR-based knockout and overexpression experiments will test its functional relevance in erythroid differentiation.

References

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