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AUTHOR'S VIEWS



Enhancer recruitment of a RUNX1, HDAC1 and TLE3 co-repressor complex by mis-expressed FOXC1 blocks differentiation in acute myeloid leukemia

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ABSTRACT

Tissue-inappropriate expression of *FOXC1* (Forkhead Box C1) in acute myeloid leukemia confers a monocyte/macrophage lineage differentiation block. We discovered that *FOXC1* interacts with RUNX1 (Runt-Related Transcription Factor 1) to stabilize a RUNX1, HDAC1 (Histone Deacetylase 1) and TLE3 (Transducin-like enhancer protein 3) repressor complex at enhancers controlling myeloid differentiation genes.

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FOXC1 (Forkhead Box C1) belongs to the Forkhead family of transcription factors, which share a conserved DNA binding domain called the Forkhead domain. As for other Forkhead proteins, *FOXC1* plays fundamental roles in development and it is a critical regulator of mesenchymal and mesodermal differentiation.¹ Specifically, *FOXC1* is required for the correct formation of the neural tube, for generation of bone and cartilage, and for development of the anterior eye segments.^{2,3} In humans, inherited haploinsufficiency of *FOXC1* causes the Axenfeld-Rieger syndrome, defined by abnormalities of the eye, hearing loss, skeletal, dental, and cardiac malformations.⁴

In addition to its frequent overexpression in a range of solid malignancies where it promotes proliferation, apoptosis resistance, hypoxia adaptation, and enhanced cellular invasion and metastasis,⁴ *FOXC1* is important in human acute myeloid leukemia (AML). *FOXC1* is mis-expressed in ~20% of AML cases where it confers a monocyte/macrophage lineage differentiation block; a block to normal differentiation is the cardinal feature of AML.⁵ While *FOXC1* is required for proper function of hematopoietic stem cell niche cells, it is neither expressed in nor required for normal function of hematopoietic cells.⁶ Further, in AML as it does in solid malignancies, high-level expression of *FOXC1* has prognostic value: patients with high *FOXC1* expression have poorer outcomes.

Given the importance of *FOXC1* in cancer and the unknown mechanism by which it confers blocked differentiation in AML, we profiled *FOXC1*'s protein interactome on chromatin by Rapid Immunoprecipitation Mass spectrometry of Endogenous protein (RIME) in patient and cell line AML samples.⁷ We discovered that RUNX1 (Runt-Related Transcription Factor 1), a critical regulator of myeloid differentiation which is frequently mutated in AML,⁸ and *FOXC1* interact through their respective DNA binding domains. In the

case of *FOXC1*, the interaction requires residues within Wing 2 of the Forkhead domain, which are mutated in the Axenfeld-Rieger Syndrome.⁹

To investigate whether *FOXC1* acts in concert with RUNX1 genome-wide, we performed chromatin immunoprecipitation (ChIP) with next-generation sequencing to identify DNA binding sites, and discovered that the two factors co-occupy ~600 primed and active enhancers in AML cells located close to genes controlling myeloid differentiation. This suggested functional collaboration of the two proteins at enhancer sites for regulation of gene expression. We analyzed the properties of these "FR" co-occupied sites and their activity upon *FOXC1* depletion. Consistent with a model whereby *FOXC1* and RUNX1 stabilize each other's association with chromatin, we determined that doubly occupied FR-bound enhancers exhibit higher levels of co-localized RUNX1, *FOXC1*, and also the co-repressor proteins HDAC1 (Histone Deacetylase 1, a known RUNX1 binding partner) and TLE3 (Transducin-like enhancer protein 3) by comparison with singly occupied sites bound by either *FOXC1* or RUNX1, but not both. Of note, Groucho-family member co-repressor TLE3 was identified as a strong interacting protein of *FOXC1* in our RIME data, and is known to be recruited to chromatin through interaction of the C-terminal WRPY motif of RUNX proteins with the central pore of the C-terminal β -propeller of Groucho. In other cellular contexts, Groucho is also recruited to chromatin through the C-terminal WRPW motif of HES (Hairy and Enhancer of Split) family proteins as well though an FxIxxIL Engrailed homology motif found in, for example, *Drosophila* Engrailed (*en*) or Goosecoid (*gsc*).¹⁰ While it remains unclear precisely how Groucho induces repression, a number of mechanisms have been proposed including promotion of closed chromatin, recruitment of histone deacetylase or direct interaction with core transcription machinery.¹⁰

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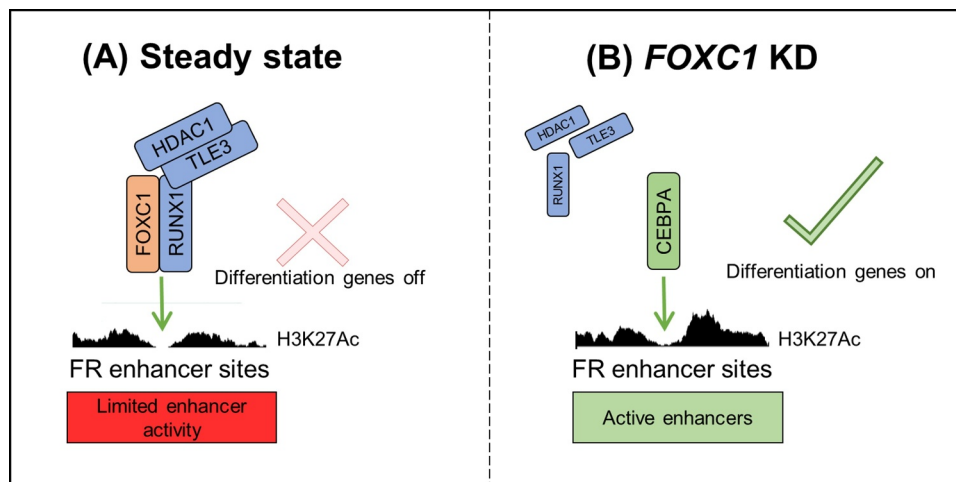


Figure 1. FOXC1 recruits a repressor complex to myeloid lineage enhancers. Depletion of FOXC1 (Forkhead Box C1) induces loss of a RUNX1 (Runt-Related Transcription Factor 1), HDAC1 (Histone Deacetylase 1) and TLE3 (Transducin-like enhancer protein 3) co-repressor complex from enhancers co-occupied by FOXC1 and RUNX1 (“FR”), permitting up regulation of a mature myeloid gene programme. (a) In steady state, “FR” enhancers are bound by FOXC1, RUNX1, HDAC1 and TLE3, creating a repressor complex that limits enhancer activity and expression of associated myeloid genes. (b) Loss of FOXC1 destabilizes the RUNX1, HDAC1 and TLE3 complex permitting ingress of CEBPA (CCAAT enhancer-binding protein alpha), increased enhancer H3K27Ac (Histone H3 lysine 27 acetylation) and increased expression of associated myeloid genes.

Following *FOXC1* knockdown – which induces leukemia cell differentiation – there was substantially greater loss of RUNX1, HDAC1 and TLE3 from FR-bound doubly occupied sites versus sites occupied by RUNX1 but not FOXC1. This is again consistent with a model whereby FOXC1 and RUNX1 stabilize each other’s association with chromatin. Furthermore, *FOXC1* knockdown also resulted in a proportion of these previously FR-bound sites recruiting CEBPA (CCAAT enhancer-binding protein alpha) leading to localized increases in surrounding H3K27 (Histone H3 lysine 27) acetylation, enhancer activation, and up regulation of nearby differentiation genes. Thus, FOXC1 serves as a transcription repressor through stabilization of RUNX1, HDAC1, and TLE3 binding to limit enhancer activity (Figure 1). Once depleted, the molecular changes induced by the lack of FOXC1 activate a myeloid gene program that pushes cells to differentiate.

Interestingly, we also noted that following *FOXC1* KD there was a wholesale redistribution of RUNX1 and TLE3 binding genome-wide which was, in general, away from enhancers and toward promoters. This included increased binding of RUNX1 and TLE3 to promoter regions of self-renewal genes such as *MYC* (*MYC* proto-oncogene, bHLH transcription factor) and *MYB* (*MYB* Proto-Oncogene, Transcription Factor). The redistributed promoter binding of RUNX1 and TLE3 associated genome-wide with down regulation of gene expression. Together these data demonstrate that sustained expression of *FOXC1* serves to prevent a differentiation cascade, which includes the redistribution of the transcription repressive function of RUNX1 from enhancers to promoters.

In conclusion, while AML is characterized by genetic heterogeneity, a differentiation block to the myeloid lineage is the pathognomonic feature of the disease. Tissue-inappropriate expression of *FOXC1* contributes to a monocytic/macrophage lineage block in ~20% of AML cases. Our data identify a repressor complex formed by interaction of FOXC1, RUNX1, HDAC1,

and TLE3 as the core component that limits activity of a critical subset of myeloid lineage enhancers thereby preventing differentiation. Our study further suggests that therapeutic targeting of the protein:protein interactions of FOXC1 with RUNX1, or RUNX1 with TLE3, in appropriate patients may potentially prove advantageous.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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