

There will be blood: hematopoiesis control by mediator subunit MED12

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Comment on: Aranda-Orgilles B, Saldaña-Meyer R, Wang E, *et al.* MED12 Regulates HSC-Specific Enhancers Independently of Mediator Kinase Activity to Control Hematopoiesis. *Cell Stem Cell* 2016;19:784-799.

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Hematopoiesis is sustained by the presence of hematopoietic stem cells (HSCs) that balance self-renewal and differentiation through controlled expression of enhancer-dependent transcriptional programs enforced by lineage-specific master transcription factors. The regulatory potential of these cardinal transcription factors is often realized with the assistance of Mediator, a conserved multiprotein interface between distal, enhancer-bound regulators and the RNA polymerase II machinery assembled on core promoters. Within Mediator, CDK8 and its partner Cyclin C (*CycC*) combine with MED12 and MED13 to form a 4-subunit kinase module that variably associates with a 26-subunit Mediator core (1). MED12, which anchors and activates *CycC*-CDK8 in Mediator, has previously been implicated in hematopoiesis through forward genetic and mutational screening in zebrafish embryos and human cancers, respectively, revealing links to both myelopoiesis and leukemia (2,3). However, the molecular basis for MED12 in these biological and pathological processes is unclear. Against this backdrop, the Aifantis laboratory recently undertook to more rigorously investigate the role of MED12 in the hematopoietic system (4).

Using several hematopoietic-specifically targeted *Med12* mouse models, including conditional *Med12* deletion in both the embryonic and adult hematopoietic systems, the authors showed that *Med12* ablation triggers a rapid loss of HSCs, likely through apoptosis, leading to acute bone marrow aplasia and rapid lethality. Reciprocal and competitive bone marrow transplantations between

conditional *Med12* knockout mice and their control littermates confirmed a cell-autonomous function for MED12 in HSC maintenance. Interestingly, genetic ablation of additional kinase module subunits, including *Med13*, *CycC*, and *Cdk8*, did not phenocopy deletion of *Med12* with respect to key HSC properties, leading the authors to deduce a kinase-independent function for MED12 in the control of HSC homeostasis. RNA profiling revealed a critical role for MED12 in enforcing a signature HSC gene expression program, characterized by high level expression of hematopoietic lineage-specific master transcription factors, while ChIP-seq identified MED12 to be a constituent component of HSC-specific enhancers and super-enhancers (SEs), where it was found particularly enriched on the latter along with hallmark histone modifications, including H3K27ac and H3K4me1. Significantly, comparative ChIP-seq in MED12-proficient and MED12-deficient HSCs revealed an unanticipated role for MED12 in maintenance of H3K27ac levels on SEs, and subsequent IP-mass spectrometry identified a likely mechanism involving interaction between MED12 and p300, an established H3K27 acetyltransferase. This scenario was subsequently validated, as the authors found that targeted depletion of MED12 in HSCs led to impaired recruitment of p300 on hematopoietic-specific SEs, indicating that reduced expression of key hematopoietic-specific genes following MED12 depletion derives from diminished levels of p300-dependent H3K27ac. Taken together, the authors conclude that

MED12 and p300 cooperate to ensure the function of lineage-specific SEs that drive high level expression of key stem cell genes required for maintenance of a balanced hematopoietic system.

This elegant and rigorously comprehensive study identifies a new role for MED12 in HSC homeostasis through epigenetic control of enhancer-dependent gene networks, and further links this activity with a novel kinase-independent function for MED12 in Mediator, compelling new discoveries with important implications for hematopoietic development and disease. First, the revelation that MED12/Mediator recruits p300 to epigenetically configure HSC enhancers to an active state clarifies the component nature and functional mechanics of the regulatory apparatus governing HSC-specific gene expression programs. This knowledge is critical to decipher the fundamental logic underlying gene expression circuits that control HSC fate and how these circuits are pathologically rewired as a course of disease, including blood cancers.

Second, this study serves to highlight the context-dependent function of MED12 in stem cell biology. Thus, as documented clearly by Aranda-Orgilles *et al.*, MED12 suppresses cell death in HSCs. By contrast, in neural stem cells, MED12 promotes G1/S phase cell cycle progression (and concomitantly suppresses cell-cell and cell-matrix interactions) with no influence on apoptotic programs (5). MED12 also appears to promote cell proliferation in epidermal progenitors, as Aranda-Orgilles *et al.* observed a reduction in Ki67 expression upon MED12 depletion in this stem cell compartment. The molecular bases underlying the observed cell type-specific functions of MED12 remain unclear, but could be dictated by the lineage-specific transcriptional regulatory and/or co-regulatory proteins with which it directly interfaces. Resolution of this issue will be important to clarify whether and how genetic variation in MED12 linked to human disease, including neurodevelopmental disorders and cancer (6), derives from its diverse biological roles in distinct stem cell compartments.

Third, this study provides the first mechanistic description of MED12 in control of SE function. In this regard, MED12 has previously been implicated as a structural and functional constituent of SEs, clustered enhancer elements that drive high-level expression of key cell identity and disease genes (7). Thus, MED12 density is a reliable indicator of functional SEs, and disruption of Mediator kinase activity leads to dysregulation of SE-

associated genes (7,8). However, a mechanistic basis for MED12 in SE function has not heretofore been described. By showing that MED12 controls the deposition of H3K27ac on SEs through direct recruitment of the H3K27-acetyltransferase p300, itself a prominent regulator of HSC function, the Aifantis laboratory provides mechanistic clarity and reveals an important intersection between genetic and epigenetic factors that dictate HSC fate through control of enhancer-driven gene expression programs.

Finally, the new findings of Aranda-Orgilles *et al.* may have important implications for understanding the role of MED12 in leukemia. MED12 is an established driver of human tumorigenesis (1), and recently, mutations in Med12 exons 1 and 2 were found in $\approx 5\%$ of chronic lymphocytic leukemias (CLL) (3). Notably, however, these CLL-linked MED12 mutations overlap with those identified at high frequency in uterine leiomyomas that are known to disrupt Mediator kinase activity (9), which Aranda-Orgilles *et al.* found to be dispensable for MED12-dependent HSC regulation. In this regard, it is notable that CycC was recently identified as a haploinsufficient tumor suppressor in T-cell acute lymphoblastic leukemia (T-ALL), wherein chromosomal deletion of CycC disrupts Mediator-associated CDK8 activity, leading to pathological stabilization of the NOTCH intracellular domain (NICD) and oncogenic NOTCH1 signaling (10). As NOTCH1 is also a prominent driver of CLL, it is possible that CLL-linked MED12 mutations similarly disrupt Mediator kinase activity, leading to oncogenic NOTCH1 signaling through pathologically stabilized NICD. Thus, whether and how oncogenic mutations in MED12 impact its HSC functions leading to CLL remains unclear. Nonetheless, the recent findings of Aranda-Orgilles *et al.* significantly advance our current understanding of the biology and functional mechanics of the regulatory apparatus that controls the expression of lineage-specific gene expression programs that inform HSC fate.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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