

# Arkadia: a new player in hematopoietic stem and progenitor cell development

Sarada Ketharnathan<sup>1</sup> and Jason N. Berman<sup>1,2</sup>

<sup>1</sup>CHEO Research Institute and <sup>2</sup>Departments of Pediatrics and Cellular and Molecular Medicine, University of Ottawa, Ottawa, Canada

**Correspondence:** J. Berman

[jberman@cheo.on.ca](mailto:jberman@cheo.on.ca)

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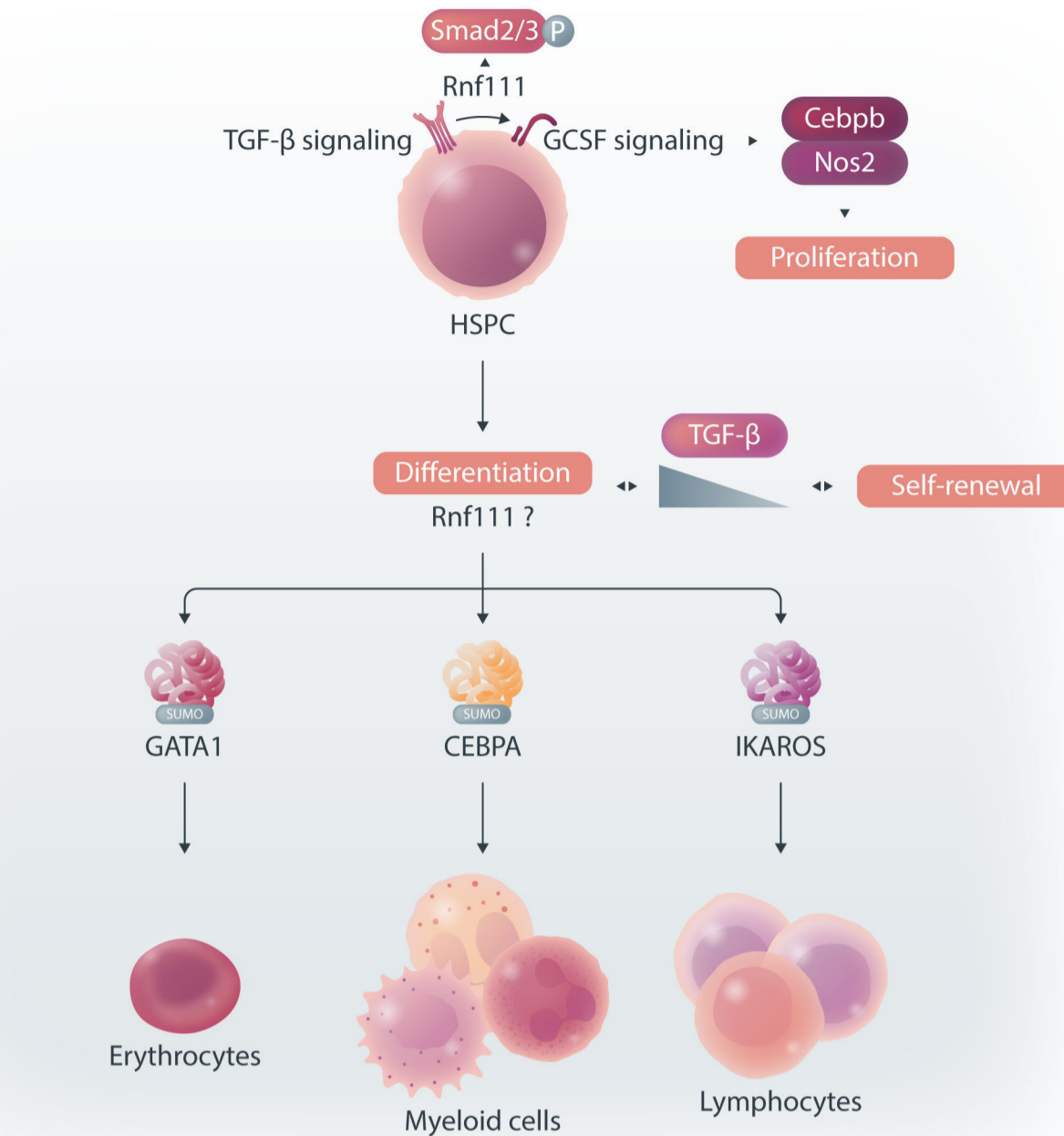


In this issue of *Hematologica*, Liu *et al.*<sup>1</sup> describe Rnf111 as a novel regulator of hematopoietic stem and progenitor cell (HSPC) development. Rnf111 maintains HSPC responsiveness to the transforming growth factor-beta (TGF- $\beta$ ) pathway by modulating the levels of downstream effectors, phosphorylated Smad2/3, and downstream granulocyte colony-stimulating factor receptor (GCSFR)/nitric oxide signaling.

Post-translational modifications regulate various aspects of protein biology including cellular distribution, function and stability. Ubiquitin and small ubiquitin-like modifier (SUMO) proteins are post-translational modifications that go through similar processing mechanisms starting with ATP-mediated activation by E1 ligases, conjugation by E2 ligases and finally binding to the target protein by E3 ligases. However, unlike ubiquitination, which almost always targets proteins for degradation, SUMOylation has multifaceted roles in modulating protein stability, localization and binding interactions. Two mammalian SUMO-targeted E3 ligases (STUBL) have been identified, namely RNF4 and Arkadia/RNF111. STUBL are unique given their abilities to interact with both ubiquitin and SUMO proteins, adding to the repertoire of protein regulatory mechanisms.<sup>2</sup> Growing evidence suggests inherent roles for SUMOylation in hematopoiesis.<sup>3,4</sup> SUMO moieties have been identified in key hematopoietic transcription factors such as GATA1, IKAROS and CEBPA. Of the two mammalian STUBL, RNF4 is well characterized with prominent functions described in DNA replication, DNA damage and mitosis.<sup>2</sup> Furthermore, an indispensable role for Rnf4 in granulopoiesis was illustrated in the zebrafish, in which failed degradation of SUMOylated Dnmt1 results in hypermethylation of the *cebpa* promoter leading to reduced granulopoiesis.<sup>5</sup> Both RNF4 and RNF111 are known to regulate the stability of the promyelocytic protein (PML) and its pathogenic fusion, PML-RAR $\alpha$ , which is frequently seen in acute promyelocytic leukemia.<sup>6</sup> SUMOylation of PML-RAR $\alpha$  is required for leukemic transformation.

In a twist of fate, treatment with standard drugs for acute promyelocytic leukemia, arsenic trioxide and all-*trans* retinoic acid, increases SUMOylation of PML-RAR $\alpha$ , making it more susceptible to degradation by STUBL. RNF111 is also a known activator of TGF- $\beta$  signaling. It binds p-Smad2/3 and degrades negative regulators of TGF- $\beta$  namely Smad7, SnoN and Ski in a SUMO-independent manner.<sup>7</sup> However, the role of RNF111 in hematopoiesis has not been previously explored before now.

Utilizing germline loss-of-function mutants and transient morphants, Liu *et al.* showed that loss of zebrafish *rnf111* results in reduced HSPC as well as downstream lineages including erythrocytes, neutrophils and lymphocytes. Unlike the STUBL-dependent functions of Rnf4 in granulopoiesis,<sup>5</sup> the authors showed that Rnf111 regulates HSPC development solely through its ubiquitin-dependent ligase function. In the absence of Rnf111, p-Smad2/3 was reduced in HSPC due to retention of the TGF- $\beta$  inhibitor, Smad7. The authors validated these findings using a drug called definitive endoderm 2 inducer (IDE2) that activates TGF- $\beta$  signaling and induces Smad2 phosphorylation. IDE2 treatment rescued the HSPC defect in Rnf111 mutants thus confirming the essential role of TGF- $\beta$  in HSPC specification. It is unclear whether these effects also extend to the rescue of HSPC differentiation. The TGF- $\beta$  pathway is complex and plays a dosage-dependent role in balancing HSPC proliferation and commitment to differentiation.<sup>8</sup> High TGF- $\beta$  expression favors quiescence whereas low expression selectively promotes expansion of myeloid-biased HSPC. The authors delved further into the molecular mechanism by which TGF- $\beta$  exerts its effects and identified that p-Smad2/3 interacts directly with the *gcsfr* promoter. Granulocyte colony-stimulating factor (GCSF) cytokine signaling promotes HSPC expansion and is also a key regulator of neutrophil differentiation. GCSF-dependent emergency granulopoiesis is driven by the transcription factor, *CEBPB*, in concert with a cytokine-inducible form of nitric oxide (*NOS2*).<sup>9</sup> Injection of *cebpb* mRNA or treatment



**Figure 1. Rnf111 mediates hematopoietic stem and progenitor cell proliferation through the transforming growth factor- $\beta$  pathway and downstream Gcsf-Cebpb-Nos2 signaling axis.** Rnf111 may have potential regulatory roles in hematopoietic stem and progenitor cell differentiation through SUMOylation of master transcription factors. Smad2/3: mothers against decapentaplegic homolog 2/3; Rnf111: ring finger protein 111; TGF- $\beta$ : transforming growth factor-beta; GCSF: granulocyte colony-stimulating factor; Cebpb: CCAAT enhancer binding protein beta; Nos2: nitric oxide synthase 2; HSPC: hematopoietic stem and progenitor cell; GATA1: GATA binding protein 1; CEBPA: CCAAT/enhancer-binding protein-alpha.

with a nitric oxide agonist partially rescued the HSPC defects in the *rnf111* mutants, providing conclusive evidence for the involvement of Cebpb-Nos2a signaling in Rnf111-mediated HSPC development.

This study by Liu *et al.* provides the first elaborate characterization of the role of Rnf111 in hematopoiesis. The study findings raise some interesting questions. First, can Rnf111 be used to modulate TGF- $\beta$  signaling and thereby control hematopoietic output? Fine-tuning TGF- $\beta$  expression can dictate states of HSPC renewal, quiescence and lineage commitment. Dissecting these mechanisms may have far-reaching therapeutic applications, for example, improving the efficiency of *ex vivo* hematopoietic stem cell expansion for transplantation or promoting bone marrow recovery after chemotherapy. TGF- $\beta$  signaling is also dysregulated in hematologic malignancies;<sup>10</sup> loss of TGF- $\beta$  is seen in leukemia either through decreased expression and/or mutation of ligands or by the loss of cell-surface receptors. In contrast, elevated TGF- $\beta$  is seen in myeloproliferative disorders. Modulating TGF- $\beta$  signaling to drive/curb hematopoietic differentiation in these conditions may provide an adjustable lever that can be exploited as a novel therapeutic target. A second question is whether the STUBL-dependent function of Rnf111 is relevant for hematopoiesis? This is something that remains to be studied. Given that many blood transcription factors are SUMOylated, it is tempting to speculate that Rnf111 may affect hematopoietic differentiation by directly regulating transcription factor stability. Alternatively, as evidenced by Rnf4-mediated granulopoiesis, ubiquitination/SUMOylation of additional players such as epigenetic modifiers may play a role in these processes.

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In sum, Liu *et al.* have effectively employed the conserved hematopoiesis, ease of genetic manipulation and whole organism phenotypic readouts of the zebrafish model to provide new insights into vertebrate hematopoiesis. Their findings have the translational potential to inform ways of manipulating hematopoietic stem cell regulation for therapeutic applications in human blood disorders.

### Disclosures

*JNB is a member of the Scientific Advisory Board for Oxford Immune Algorithmics. SK has no conflicts of interest to disclose.*

### Contributions

*SK wrote the editorial and generated the figure. JNB revised and edited the editorial and the figure.*

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