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by Sarada Ketharnathan and Jason N. Berman

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

Sarada Ketharnathan[1] and Jason N. Berman[1,2]

[1]CHEO Research Institute, Ottawa, Canada; [2]Departments of Pediatrics and Cellular and Molecular Medicine, University of Ottawa, Ottawa, Canada.

Correspondences to: Jason Berman, Chief Executive Officer and Scientific Director, CHEO Research Institute, 401 Smyth Road, Ottawa (ON) K1H 8L1

Tel: (613) 737-7600 x 2957

jberman@cheo.on.ca

In this issue of Hematologica, Liu *et al.*[1] describe Rnf111 as a novel regulator of hematopoietic stem and progenitor cell (HSPC) development. Rnf111 maintains HSPC responsiveness to the TGF-β pathway by modulating the levels of downstream effectors, phosphorylated Smad2/3, and downstream Gcsfr/nitric oxide signaling.

Post-translational modifications (PTMs) regulate various aspects of protein biology including cellular distribution, function and stability. Ubiquitin and small ubiquitin-like modifier (SUMO) are PTMs 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 that almost always targets proteins for degradation, SUMOylation has multifaceted roles in modulating protein stability, localization and binding interactions. Two mammalian SUMO-targeted E3 ligases (STUBLs) have been identified namely, RNF4 and Arkadia/RNF111. STUBLs are unique given their abilities to interact with both ubiquitin and SUMO proteins, adding to the repertoire of protein regulatory mechanisms[2]. Growing evidence suggests inherent roles for SUMOylation in hematopoiesis[3,4]. SUMO moieties have been identified in key hematopoietic transcription factors such as GATA1, IKAROS and CEBPA. Of the two mammalian STUBLs, RNF4 is well characterized with prominent functions described in DNA replication, DNA damage and mitosis[2]. Further, an indispensable role for Rnf4 in granulopoiesis was illustrated in the zebrafish, where failed degradation of SUMOylated Dnmt1 results in hypermethylation of the *cebpa* promoter leading to reduced granulopoiesis [5]. Both RNF4 and RNF111 are known to regulate the stability of the promyelocytic protein (PML) and its pathogenic fusion, PML-RARα, that is frequently seen in acute promyelocytic leukemia (PML)[6]. SUMOylation of PML-RARα is required for leukemic transformation. In a twist of fate, treatment with standard PML treatments, arsenic trioxide and all-trans retinoic acid, increases SUMOylation of PML-RARα, making it more susceptible to degradation by STUBLs. RNF111 is also a known activator of TGF-β signaling. It binds p-Smad2/3 and degrades negative regulators of TGF-β namely, Smad7, SnoN and Ski in a SUMO-independent manner[7]. 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*. show that loss of zebrafish *rnf111* results in reduced HSPCs as well as downstream lineages including erythrocytes, neutrophils and lymphocytes. Unlike the STUBL-dependent functions of Rnf4 in granulopoiesis[5], 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 HSPCs due to retention of the TGF-β inhibitor, Smad7. The authors validated these findings using a drug called definitive endoderm 2 inducer (IDE2) that activates TGF-β signaling and induces Smad2 phosphorylation. IDE2 treatment rescued the HSPC defect in Rnf111 mutants thus confirming the essential role of TGF-β in HSPC specification. It is unclear if these effects also extend to the rescue of HSPC differentiation. The TGF-β pathway is complex and plays a dosage-dependent role in balancing HSPC proliferation and commitment to differentiation[8]. High TGF-β expression favors quiescence whereas low expression selectively promotes expansion of myeloid-biased HSPCs. The authors delved further into the molecular mechanism by which TGF-β exerts its effects and identified that p-Smad2/3 directly interacts with the *granulocyte colony-stimulating factor receptor (gcsfr)* promoter. 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*)[9]. Injection of *cebpb* mRNA or treatment 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: Can Rnf111 be used to modulate TGF-β signaling and thereby control hematopoietic output? Fine-tuning TGF-β 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 HSC expansion for transplantation or promoting bone marrow recovery post-chemotherapy. TGF-β signaling is also dysregulated in hematological malignancies[10]; loss of TGF-β 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-β is seen in myeloproliferative disorders. Modulating TGF-β 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? 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.

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 HSC regulation for therapeutic applications in human blood disorders.

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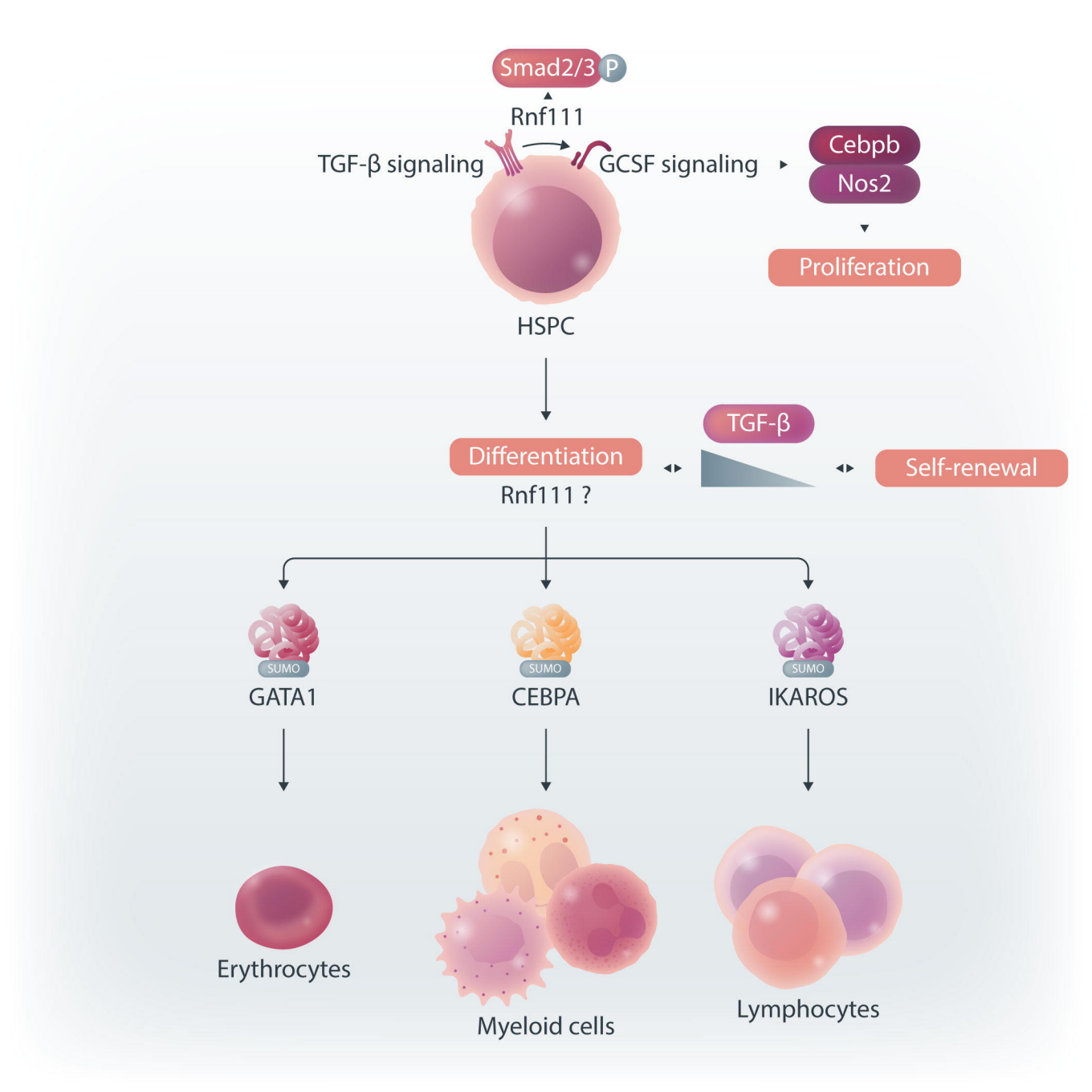
Figure. Rnf111 mediates HSPC proliferation through the TGF-β pathway and downstream Gcsf-Cebpb-Nos2 signaling axis. Rnf111 may have potential regulatory roles in HSPC differentiation through SUMOylation of master transcription factors. HSPC: hematopoietic stem and progenitor cell.


Smad2/3 P
Rnf111
TGF-β signaling
GCSF signaling
Cebpb
Nos2
Proliferation
HSPC
Differentiation
Rnf111 ?
TGF-β
Self-renewal
SUMO
GATA1
SUMO
CEBPA
SUMO
IKAROS
Erythrocytes
Myeloid cells
Lymphocytes