Twist1 as a pivotal regulator of hematopoietic stem cell fate

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Hematopoietic stem cells (HSCs) are characterized by their capacity to maintain lifelong production of mature blood cells, and to repopulate the hematopoietic system in response to hematopoietic stresses.[1] Numerous studies have revealed phenotypic and functional HSC heterogeneity, with evidence for the presence of lineage (myeloid-, lymphoid-, megakaryocytic)-biased HSCs within the HSC compartment.[2] However, little is known about how lineage-biased HSCs are maintained and regulated under steady-state and emergency hematopoiesis. In the latest issue of Blood, Wang et al demonstrate an essential role for TWIST1 in maintaining lymphoid-biased (Ly-biased) HSC pool under both steady-state and stress conditions, and provide a novel molecular underlying mechanism of HSC maintenance. They found that TWIST1 preserves the function of HSCs by preventing hyperactivation of the CACNA1B/Ca^{2+}/mitochondria axis under steady-state and stress conditions.[3]

TWIST1 is a highly conserved basic helix-loop-helix transcription factor essential for the development of mesoderm-derived tissues.[4] In humans, TWIST1 has been found to be overexpressed in various solid tumors,[5] and further studies imply that it plays a critical role in promoting tumor epithelial-mesenchymal transition, metastasis, and drug resistance.[6] Additionally, TWIST1 has attracted intense interest due to its contribution to the generation and maintenance of cancer stem cells. For example, overexpression of TWIST1 in breast cell lines, head and neck squamous cell carcinoma cells, and cervical cancer cells enhanced tumor-initiating and self-renewal capability.[7] In the hematopoietic system, TWIST1 is highly enriched in HSCs. *Twist1* overexpression promotes HSCs self-renewal and lineage commitment.[8] However, the function of TWIST1 in HSC maintenance has not been well studied. Using a hematopoietic-specific conditional *Twist1*-knockout mouse model, Wang et al performed an extensive characterization of the HSC compartment. They found that loss of *Twist1* significantly reduced pool size, dormancy and self-renewal capacities of HSC, and skewed myeloid differentiation in steady-state hematopoiesis, suggesting its essential role in HSC regulation.[3]

Intriguingly, the authors demonstrated that HSC reduction occurs mainly in Ly-biased HSCs, indicating that enhanced myeloid lineage differentiation in *Twist1* cKO mice is attributed likely to the loss of lymphoid-biased HSCs. The finding is consistent with the notion of a pre-determined biased differentiation predisposition. Notably, the frequencies of Ly-biased HSCs in *Twist1* cKO mice were also remarkably decreased upon both irradiation (IR) and 5-fluorouracil (5-FU) treatments.[3] During stress hematopoiesis, lineage-biased HSCs prominently emerge due to inflammation or recovery from myeloablation.[9] A recent study has revealed that HSC heterogeneity may be associated with HSC metabolic state.[10] Wang et al further found that *Twist1* deletion increased intracellular and mitochondrial Ca^{2+} concentration, mitochondrial membrane potential, and glucose uptake in Ly-biased HSCs, but not in myeloid-biased HSCs upon IR stress. These important results suggest that TWIST1 can cause functional heterogeneity within the HSC pool, supporting the notion that mitochondrial metabolic state is a determinant for HSC heterogeneity. MFN2 is necessary for maintaining lymphoid-biased HSCs through calcium-dependent signaling,[10] and consistently, the authors found reduced expression of MFN2 in *Twist1*-deficient HSCs following 5-FU treatment.[3] Together, the authors demonstrate that TWIST1 is required for the preservation of Ly-biased HSCs, providing novel molecular insights into the basis of HSC heterogeneity.

In embryonic and human mesenchymal stem cells, calcium entry into the cytoplasm from the extracellular space is mediated mainly by store-operated Ca^{2+} channels rather than the voltage-gated calcium channels (VGCCs) or by the Na^{+}/Ca^{2+} exchangers.[11] The mechanism of calcium entry seems to be different in HSCs. A previous study found that suppression of intracellular Ca^{2+} level into HSCs by Nifedipine (blockers of L-type voltage-gated Ca^{2+} channels) abolished the resultant mitochondrial activation and prolonged cell division interval in HSCs, and simultaneously achieved both cell division and HSC maintenance.[12] However, this study has not shown how the intracellular transfer of Ca^{2+} occurred. In search for mechanisms underlying the effect of TWIST1 on HSCs upon stress, the authors performed an integrative analysis of ATAC-sequencing and RNA-sequencing data and found that the expression levels of the members of the VGCC family calcium channel a1b subunit (*Cacna1b*) were most elevated in *Twist1*-deleted HSCs after IR treatment. Furthermore, the expression of *Cacna1b* was downregulated in murine LT-HSCs and but upregulated with differentiation, suggesting an inverse relationship between *Cacna1b* and *Twist1* expression in HSCs. They further demonstrated that TWIST1 is a direct transcriptional repressor of CACNA1B under steady-state

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and stress conditions.[3] CACNA1B which encodes the N-type voltage-gated Ca^{2+} channels, is highly expressed in the brain and peripheral nervous system and important for regulating neuropathic pain.[13] Little is known about its role in HSCs. Thus, Wang et al provide new evidence of HSC maintenance regulated by the TWIST1-CACNA1B axis.

Ca^{2+}, a key intracellular secondary messenger, is a crucial dynamic regulator of mitochondrial metabolism, apoptosis, and proliferation.[14] Specific roles of calcium in HSC maintenance mechanisms are only beginning to be explored. It is well recognized that HSCs display lower levels of intracellular calcium compared with progenitors conveyed by elevated expression and activity of plasma membrane calcium efflux pumps. Low Ca^{2+} suppresses mitochondrial respiration and enhances HSC maintenance in vitro.[15] Another study demonstrated that the appropriate suppression of the Ca^{2+}-mitochondria pathway is necessary for HSC self-renewal division in vitro.[12] In line with these results, Wang et al reported that *Twist1* deletion caused a significantly increased Ca^{2+} influx into their cytosol and mitochondria, which in turn enhanced mitochondrial function. Importantly, suppression of VGCCs by Cilnidipine rescued the phenotypic and functional defects in *Twist1*-deleted HSCs under both steady-state and stress conditions in vivo. Thus, the authors highlight the importance of low intracellular Ca^{2+} in HSC maintenance under both steady-state and stress conditions.[3]

Elucidating mechanisms that regulate HSC fate control are a central focus of ongoing research. The significant aspects of this study establish the mechanistic links between the transcriptional network, calcium regulation, mitochondrial activation, and HSC fate decision. Moreover, the study offers an exciting possibility that fine-tuning of intracellular Ca^{2+} may represent a potential therapeutic strategy for relieving damaged HSCs and maintaining HSC functions.

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