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501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

Deciphering the Differential Impact of Thrombopoietin/MPL Signaling on Hematopoietic Stem Cell Function in Bone Marrow and Spleen

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Introduction

Thrombopoietin (TPO) and its receptor MPL are key regulators of hematopoietic stem cell (HSC) function. In humans, ablation of either TPO or MPL leads to bone marrow failure in the early years of life. In contrast, mice lacking TPO or MPL display marked thrombocytopenia and severely reduced HSC numbers but manage to maintain normal survival up to one year of age. In this study, we investigated whether these mice eventually develop marrow failure with longer follow up, with a specific focus on understanding the effects of TPO/MPL signaling on HSC regulation in different hematopoietic niches.

Methods

Marrow and spleen hematopoiesis in the TPO $^{-/-}$ mice and MPL $^{-/-}$ mice were examined during a 2-yr follow-up.

Results

At 2 yrs of age, TPO $^{-/-}$ and MPL $^{-/-}$ mice exhibited not only a severely decreased platelet count (TPO $^{-/-}$ vs. MPL $^{-/-}$ vs. control: 38 vs. 107 vs. 1255 x 10 ^{9}/L) but also a moderate reduction in neutrophil count compared to age-matched wild-type controls (TPO $^{-/-}$ vs. MPL $^{-/-}$ vs. control: 1.1 vs. 1.7vs.3.3 x 10 ^{3}/ul). Femur marrow cell count or spleen cell count was not significantly different between TPO $^{-/-}$ or MPL $^{-/-}$ mice and control mice. There was no significant difference in overall survival between the TPO $^{-/-}$ or MPL $^{-/-}$ mice and wild-type controls.

Marrow Lin $^{-}$cKit $^{+}$Sca1 $^{+}$CD150 $^{+}$CD48 $^{-}$ HSC cell numbers were significantly decreased in young (4-6mo) TPO $^{-/-}$ and MPL $^{-/-}$ mice (~4-fold) compared to age-matched control mice. This HSC loss was further worsened in old (~24mo) TPO $^{-/-}$ (25-fold) and MPL $^{-/-}$ (12-fold) mice compared to control mice. In contrast, there was no significant difference in splenic HSC cell numbers between young TPO $^{-/-}$ or MPL $^{-/-}$ mice and control mice, with only a moderate decrease of HSCs in old TPO $^{-/-}$ and MPL $^{-/-}$ (~4-fold) mice compared to control mice. *I n vivo* using BrdU labeling revealed an increased proliferation of marrow HSCs in TPO $^{-/-}$ and MPL $^{-/-}$ mice compared to age-matched control mice. In contrast, there was no significant increase in splenic HSC proliferation in TPO $^{-/-}$ or MPL $^{-/-}$ mice. These findings suggest that TPO/MPL signaling has distinct effects on both the number and the function of HSCs in the marrow and spleen.

Competitive repopulation assays revealed that HSCs from 2yr old TPO $^{-/-}$ or MPL $^{-/-}$ marrow failed to engraft the recipient mice at 12 weeks post-transplantation. We also conducted competitive repopulation assays in which spleen cells from old TPO $^{-/-}$ mice (CD45.2) were injected together with spleen cells from 2yr old wild-type competitor mice (CD45.1) into lethally irradiated wild-type recipients (CD45.1). At 12 days after transplantation, the TPO $^{-/-}$ spleen donor showed comparable engraftment in the Lin $^{-}$cKit $^{+}$Sca1 $^{+}$ stem/progenitor cell compartment, similar to the wild-type competitor spleen donor, suggesting that at least the short-term engraftment capacity of the TPO $^{-/-}$ spleen donor was not impaired. We are currently conducting further investigation to assess the long-term repopulation capacity of TPO $^{-/-}$ spleen HSCs.

To investigate how TPO/MPL ablation impacts the hematopoietic niche in the marrow and spleen, we used quantitative flow cytometry and/or fluorescence imaging to examine these niche cells and their expression of niche factors. First, while there was no significant difference in the marrow vascular area (measured by *in vivo* VE-cadherin labeling) between TPO $^{-/-}$ or MPL $^{-/-}$ mice and control mice, we observed a significant increase in the splenic vascular area in TPO $^{-/-}$ and MPL $^{-/-}$ mice. Second, CXCL12 levels were significantly decreased in marrow endothelial cells (ECs; CD45 $^{-}$CD31 $^{+}$) from TPO $^{-/-}$ and MPL $^{-/-}$ mice compared to control mice; in contrast, CXCL12 levels were significantly increased in splenic ECs from TPO $^{-/-}$ and MPL $^{-/-}$ mice. Similar results were also observed with marrow and splenic perivascular stromal cells (CD45 $^{-}$CD31 $^{-}$Ter119 $^{-}$Sca1 $^{-}$). Therefore, TPO/MPL ablation enhanced both the quantity and quality of the splenic hematopoietic niche, which could contribute to the maintenance of splenic hematopoiesis in TPO $^{-/-}$ and MPL $^{-/-}$ mice.

Last, consistent with findings from the TPO/MPL ablated mice, exogenous TPO treatment increased marrow HSC proliferation but did not alter splenic HSC proliferation. In addition, TPO treatment increased CXCL12 expression in marrow ECs, but decreased CXCL12 levels in splenic ECs.
Taken together, our study revealed the complexity of TPO/MPL signaling in HSC regulation which differs between the marrow and spleen

Disclosures No relevant conflicts of interest to declare.

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