GLUCOCORTICOID-INDUCED LEUCINE ZIPPER IS REQUIRED FOR HEMATOPOIETIC STEM CELL FUNCTION

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Introduction: Hematopoietic stem cells (HSC) activity is critical for life-long production of hematopoietic cells. The long-term HSC maintenance is associated with a quiescent state, while increase in HSC proliferation caused by extrinsic and intrinsic factors was shown to impair the HSC function. The endogenous glucocorticoid hormones (GC) control HSC proliferation and homing via regulation of the CXCL12-CXCR4chemokine axis. Various stresses may lead to the alteration in this regulatory network, thus affecting HSC function. Moreover, pharmacologic doses of synthetic GC induce apoptosis in lymphocytes as well as early lymphoid progenitors. Nevertheless, the effect of GC on survival, proliferation and lineage commitment of the most primitive HSCs are not yet well characterized. GILZ (Glucocorticoid-Induced Leucine Zipper) gene is rapidly induced by GC and mediates many of its effects, including regulation of cell growth and differentiation. We have found that gilz mRNA is expressed in HSC and progenitor cells (HPC) and is downregulated in myeloid progenitor cells.

Methods: We have addressed the role of GILZ in HSC homeostasis using GILZ knock-out (KO) mice. Generally, bone marrow samples were analyzed using flow cytometry; differential gene expression was analyzed by RNASeq method; statistical analysis were performed using GraphPad Prism.

Results: Under steady state, young GILZ-KO mice show a significant decrease in HSC and an increase in HPC frequency, while the number of HSC remains unchanged. Competitive repopulation studies using wild-type (WT) and GILZ-KO bone marrow cells (CD45.2+ allotype) along with CD45.1+ WT helper cells revealed a significant decrease in the frequency of GILZ-KO compared to WT CD45.2+ cells in the bone marrows of radiation chimeras, suggesting that GILZ-deficient HSCs have competitive disadvantage compared to WT cells. Consistently, the number of HSC was significantly decreased in the bone marrows of GILZ-KO-transplanted mice, suggesting that GILZ is required for the proper control of the HCS number in the bone marrow. In order to demonstrate that the lack of GILZ is associated with decreased HSC function, we have performed 5-FU treatment experiments in mice transplanted with WT or GILZ KO bone marrow cells to ensure the hematopoietic cell intrinsic effect of GILZ deletion on mice survival. Mice bearing GILZ KO hematopoietic cells showed significantly earlier mortality caused by the 5-FU treatment compared to mice reconstituted with WT cells. To analyze the mechanism of the defect in HSC function upon GILZ deletion, we performed comprehensive analysis of gene expression by RNAseq in purified WT and GILZ KO HSCs. We identified several deregulated cellular pathways implicated in HSC maintenance and function.

Conclusions: Altogether, these data identify GILZ is a novel critical regulator of HSCs function.