Glucocorticoid induced leucine zipper (GILZ) contributes to HSC homeostasis in mice

T. Frammartino[1], M. Cimino[1], D. Sorcini[1], R. Lupo[1], F. De Chirico[1], O. Bereshechenko[1], S. Bruscoli[1], C. Riccardi[1].
Dept. of Medicine, Section of Pharmacology, University of Perugia, Italy

Blood cells derive from a hierarchically organized process that begin from the hematopoietic stem cells (HSC) that possess the properties of both multi-potency and self-renewal.
Under homeostatic conditions mature blood cells are produced from HSC that reside in specialized niches in the bone marrow (BM) cavity and cycle rarely. Increased HSC cycling is associated with loss of self-renewal capacity. Thus This conditionHSC quiescence is necessary to maintain the pool of HSC during lifetime of the organism to meet its demand for continuous replenishment of mature blood cells.
Glucocorticoids (GC) are hormones that regulate many cellular functions, such as cell growth, survival and differentiation and mediate inflammatory and immune suppression; synthetic GC are widely used as therapeutic agents. It has been proposed that Dexamethasone increases the quiescence of HSC by upregulating the levels of Ang-1 in mouse bone marrow, however, the effect of GC on survival, proliferation and lineage commitment of the most primitive HSC are not yet defined.
GILZ (Glucocorticoid-Induced Leucine Zipper), which expression is upregulated by GC(2), contributes to the regulation of cell growth and differentiation by inhibiting Ras/MAPK pathway(3) and NF-kB activity(4). These pathways are important for HSC homeostasis, therefore GILZ represents an attractive candidate for functional validation of its role in hematopoietic system.
We have isolated HSC subpopulation by cell sorting and found that*gilz*mRNA is expressed at higher levels in the most primitive quiescent hematopoietic stem cells and less in the proliferating progenitors populations defined by the markers CD150 and CD48.
To address the role of GILZ on normal HSC and progenitor cell homeostasis we have used GILZ knock-out (KO) mice. Under steady state, young GILZ KO mice show a decrease in the frequency of most primitive HSC (defined as $Lineage^{-}Sca1^{+}c\text{-}Kit^{+}CD150^{+}FLT3^{-}CD48^{-}$). Cell cycle analysis of freshly isolated bone marrow from GILZ KO mice show evidence of increased cell cycling as demonstrated by Ki-67 staining. In order to verify whether the phenotype observed in GILZ KO mice was hematopoietic lineage-intrinsic, we performed competitive repopulation assay by injecting bone marrow cells isolated from wild type or GILZ KO mice (CD45.2 allotype) together with a wild type competitor cells (CD45.1 allotype) into lethal irradiated recipient mice (CD45.1/2 allotype). The blood of competitively transplanted mice revealed transient overrepresentation of donor-derived GILZ KO cells compared to wild type cells at 12 weeks after transplantation. However, there was a subsequent drop in the frequency of GILZ KO cells in peripheral blood at one year time-point. Coherently, bone marrows of these mice revealed a drop in the frequency and number of GILZ-deficient LSK, as well as LT-HSC, suggesting that GILZ plays a role in HSC maintenance.
It has been demonstrated that GILZ inhibits MAP-kinase/ERK-kinase signaling pathways by interaction with Raf-1 and inhibits NF-kB transcriptional activity. For these reasons we will investigate whether these mechanisms contribute to the increased proliferation in GILZ KO HSC.

1. D'Adamio F, Zollo O, Moraca R, Ayroldi E, Bruscoli S, Bartoli A, et al. A new dexamethasone-induced gene of the leucine zipper family protects T lymphocytes from TCR/CD3-activated cell death. Immunity. dicembre 1997;7(6):803–12.
2. Ayroldi E, Zollo O, Bastianelli A, Marchetti C, Agostini M, Di Virgilio R, et al. GILZ mediates the antiproliferative activity of glucocorticoids by negative regulation of Ras signaling. J Clin Invest. giugno 2007;117(6):1605–15.
3. Ayroldi E, Migliorati G, Bruscoli S, Marchetti C, Zollo O, Cannarile L, et al. Modulation of T-cell activation by the glucocorticoid-induced leucine zipper factor via inhibition of nuclear factor kappaB. Blood. 1 agosto 2001;98(3):743–53.