Glucocorticoid-Induced Leucine Zipper (GILZ) regulates hematopoietic stem cell function and tumor development in a mouse model of CEBPA mutant AML

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Acute myeloid leukemia (AML) is the most common acute leukemia in adults. Mutations in the *CEBPA* gene are found in 9-12% of AML cases and are divided into two major groups: N-terminal mutations that block the translation of the growth suppressing 42kDa isoform, and C-terminal mutations that generate inframe insertions/deletions within the basic region-leucine zipper DNA binding domain. Majority of AML cases with *CEBPA* mutations bear both types of mutation on separate alleles, indicating that these cooperate in leukemogenesis. Combining of N- and C-mutations in mice results in loss of HSC quiescence and expansion of premalignant pool of cells, associated with accelerated AML development. Since hematopoietic stem cells (HSCs) expansion was associated with faster AML progression, its elimination may represent a way to prevent tumor development. Cell cycle analysis has demonstrated that mutant HSCs have increased cycling, suggesting they may be targeted by chemotherapy. The quiescent fraction of leukemogenic HSC may prove resistance and tumor relapse. For this reason molecular characterization of the resistant HSCs is necessary to find novel ways for their elimination.

Glucocorticoids (GCs) are hormones produced in response to various types of stress, including inflammation. As potent immunosuppressors, they are widely used for treating immune system diseases, as well as in organ and bone marrow transplantation. They are also used to treat patients suffering from a wide range of cancers, including hematologic malignancies. AML is considered relatively more resistant to GC action; however improved outcome of AML has been reported for the combination of chemotherapy and steroids in different AML subtypes. Glucocorticoid-induced leucine zipper (GILZ) mediates several anti-inflammatory effects of GCs, including suppression of cell growth and regulation of cell differentiation. It inhibits normal and tumor cell growth by interfering with Ras/MAPK pathway and NFKB activity. It represents therefore an attractive candidate for functional validation of its role in leukemogenesis, due to its reported tumor suppressive activity, existing functional link to *CEBPA* and possibility of modulation by available inexpensive drug. The principal aim of this project is to evaluate the effect of GC and its target GILZ on pre-AML condition in *CEBPA* mutant model of AML and to evaluate their impact on long-term leukemogenesis.

We have analyzed the effect of GILZ deficiency on HSC engraftment, myeloid differentiation and AML development in mice with compound CEBPA and GILZ KO mutant genotype in hematopoietic system. Fetal livers with *CEBPA* N/C GILZ Y/- genotypes (CD45.2+) were collected and transplanted along with wild type competitor bone marrow cells (CD45.1+) into lethally irradiated hosts. We demonstrate that when the absence of GILZ is combined with leukemogenic *CEBPA* mutations, GILZ deficiency dramatically affects the number of engrafting *CEBPA* mutant HSCs, as evidenced by the analysis of the frequency of CD45.2+ cells in peripheral blood and bone marrow of CEBPA N/C and CEBPA N/C GILZ KO fetal liver transplanted mice. Moreover, GILZ deficiency rescued the block of myeloid differentiation caused by biallelic *CEBPA* mutations, as the frequency of Mac-1+ cells was higher in CEBPA N/C GILZ KO mice compared to CEBPA N/C mice. This suggests that GILZ regulates the function of C/EBPα and/or C/EBPβ family members in normal and malignant myelopoiesis. Importantly, mice transplanted with *CEBPA* N/C GILZ Y/- showed delayed tumor development. Overall these data unravel a novel player in the regulation of normal and malignant myelopoiesis with a potential for therapeutic exploration.