**GLUCOCORTICOID-INDUCED LEUCINE ZIPPER (GILZ) REGULATES TUMOR DEVELOPMENT IN A MOUSE MODEL OF CEBPA MUTANT ACUTE MYELOID LEUKEMIA.**

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Acute myeloid leukemia (AML) is the most common acute leukemia in adults. It progresses through a distinct steps of developments associated with acquisition of specific genetic and epigenetic hits. Recent evidence demonstrates that original pre-leukemic clones are found in the end-stage tumors, and their resistance to conventional therapies may underlie the subsequent tumor relapse. Therefore, understanding the mechanisms regulating the expansion of the pre-leukemic cells is an important step to achieve their pharmacologic targeting with conventional or novel therapies. 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 growth suppressive function of CEBPa and C-terminal mutations that alter its 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. Therefore, this mouse model represents a unique system to identify the regulators of pre-leukemic HSC expansion by genetic and pharmacologic means.

Glucocorticoids (GCs) are hormones produced in response to various types of stress, including inflammation. 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 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 the fact that its expression levels are decreased in proliferating CEBPA-mutant HSCs.

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. For this purpose, radiation chiemeras were generated by transplantation of the fetal livers (embryonic day 14,5) with CEBPA N/C GILZ Y/- compound genotype (CD45.2+ allotype) along with the wild type helper bone marrow cells (CD45.1+ allotype) into lethally irradiated hosts (CD45.1/2+ allotype). We demonstrate that GILZ deficiency dramatically affects the number of CEBPA mutant HSCs, as evidenced by 1) the analysis of the frequency of CD45.2+ cells in peripheral blood of mice transplanted with CEBPA N/C and CEBPA N/C GILZ Y/- cells, 2) analysis of the HSC number in the bone marrow. GILZ deficiency also altered 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. Importantly, the decrease in the number of pre-leukemic HSCs was associated with a significant delay in tumor development, suggesting

that GILZ is required for pre-leukemic HSCs function and AML progression. Overall, these data suggest that GILZ regulates the function of CEBPA-mutant cells in malignant myelopoiesis, and unravel a novel player in the regulation of normal and malignant myelopoiesis with a potential for therapeutic exploration.