Abstract: P1289

Title: A MURINE NON-CONDITIONED BONE MARROW TRANSPLANTATION MODEL TO INVESTIGATE CLONAL HEMATOPOIESIS AND MYELOID MALIGNANCIES

Abstract Type: Poster Presentation

Topic: Stem cell transplantation - Experimental

Background:

Clonal hematopoiesis of indeterminate potential (CHIP) is characterized by the presence of mutations associated with hematological malignancies in blood or bone marrow (BM) cells, in the absence of a diagnosed hematological disorder. CHIP is associated with an increased risk of developing hematological malignancies and other age-related diseases. Bone marrow transplantation (BMT) of cells carrying CHIP mutations into irradiated mice is a useful procedure to investigate the dynamics of clonal expansion and disease progression. However, it has been documented that irradiation induces long-lasting effects in several organs, including the BM niche, which might create confounding effects. Conversely, non-conditioned transplantation models offer an alternative method to study normal and malignant hematopoiesis.

Aims:

The aim of this study was to establish and characterize a non-conditioned BMT model to overcome the unwanted effects of irradiation to study CHIP and myeloid malignancies.

Methods:

Conditional *Tet2* deletion was employed to obtain *Tet2-/-* cells from donor mice. Total BM cells containing different numbers of *Tet2-/-* or *Tet2+/+* BM cells (CD45.2+) were transplanted into C57BL/6J-*KitW-41J*/J recipients (CD45.1+), and longitudinal and terminal analyses were performed 10 months post-BMT. Flow cytometry was used to quantify CD45.2+ cells in peripheral blood (PB) and to analyze hematopoietic cell frequencies in PB and BM. Complete blood cell counts were obtained every month post-BMT using a veterinary hematoanalyzer, and histological examination of BM, spleen, and liver sections was conducted at terminal analysis.

Results:

C57BL/6J-*KitW-41J*/J (referred to as W41) mice have a point mutation in the*c-Kit* locus, which leads to partial loss of the Kit activity in hematopoietic stem cells, providing a competitive advantage for donor cells to engraft with minimal or no irradiation. In this study, we have shown that W41 mice can be used for BMT procedures without myeloablative pre-conditioning. To establish a CHIP model, 5x106 BM cells, including 5% *Tet2-/- or Tet2+/+* BM cells, were transplanted into non-conditioned W41 mice. Longitudinal analysis over 10 months post-BMT showed engraftment and gradual expansion of *Tet2-/-* cells, with no significant changes in blood counts compared to *Tet2+/+* recipients. Ten months post-BMT, no significant differences were observed in the proportion of major immune cell populations in PB or BM, neither in the histological examinations of spleen, liver and BM between *Tet2-/-* and *Tet2+/+* recipients. Flow cytometry analysis of BM revealed comparable frequencies of stem and progenitor cells between the two groups. Altogether, in this model we induced a stable and continuous expansion of *Tet2-/-* cells that did not significantly affect the normal hematopoietic system, resembling *Tet2*-driven CHIP. To generate a myeloid malignancy model, 1x106*Tet2+/+* or *Tet2−/−* BM cells were transplanted into non-conditioned W41 recipients. Increasing the numbers of transplanted *Tet2-/-* cells led to hematopoietic abnormalities, such as a significant expansion of myeloid cells and a reduction of the lymphoid compartment in PB and BM, increased proportion of myeloid progenitors in BM and hepatosplenomegaly. Altogether, this phenotype resembled a myeloproliferative disorder.

Summary/Conclusion:

The non-conditioned BMT model using W41 recipients recapitulated *Tet2*-driven clonal hematopoiesis and myeloid malignancies. This model represents a valuable tool to understand the mechanisms underlying CHIP and malignant hematopoiesis without confounding effect from myeloablative procedures.

Keywords: Bone marrow transplant, Mouse model, Irradiation, Clonal hematopoiesis of indeterminate potential