A MOUSE 5-FLUOROURACIL-BASED SUBMYELOABLATION MODEL FOR STUDYING BONE MARROW-DERIVED CELL TRAFFICKING IN REPRODUCTION

R. Tal, Y. Liu, N. Pluchino, S. Shaikh, R. Mamillapalli, H. Taylor
Department of Obstetrics, Gynecology & Reproductive Sciences, Yale School of Medicine, USA

Objective: Bone marrow (BM)-derived cells (BMDCs) contribute to endometrial regeneration. Most animal models to date investigating BMDCs recruitment to the uterus have utilized myeloablation by irradiation prior to bone marrow transplant (BMT), which leads to ovarian failure. Therefore, such models cannot be used to gain important insight into the role of BMDCs in various reproductive processes such as pregnancy. 5-fluorouracil (5-FU) is non-gonadotoxic but is associated with very low BM engraftment (3%) when given as single dose. Our objective was to develop a non-gonadotoxic mouse BMT model using 5-FU for the study of BMDCs trafficking in reproduction.
Materials & Methods: 6 weeks-old female C57BL/6J wild-type mice (n=12/group) received intraperitoneally either a single (150mg/Kg) 5-FU dose 24h before BMT (CT1), or paired-dose 5-FU with stem cell factor (SCF) 150ug/Kg on days -5 and -1 prior to BMT (CT2+SCF). Control mice received BMT or saline. For BMT, 20×10^6 unfractionated BM cells obtained from transgenic green-fluorescent protein (GFP) male mice by flushing the femur and tibia, were injected intravenously into female recipients. For fertility experiment, mice were mated on day 28 post-BMT. In a separate experiment (n=10/group), mice were sacrificed 1 month following BMT. Peripheral blood or cell suspension of minced uterine tissues were subjected to flow cytometry. Alternatively, uterine tissues were fixed in paraformaldehyde for immunohistochemistry using antibodies against CD31, CD45, cytokeratin and GFP, and assessed for colocalization under confocal microscopy. Statistical analysis was performed using t-test or Kruskal-Wallis nonparametric tests.
Results: Pregnancy rate in the CT2+SCF (90%), CT1 (90%) and BMT only (82%) was comparable to the control saline mice (92%). In addition, the number of implantation sites, number of viable fetuses, and pregnancy resorption rate was comparable in all groups. CT2+SCF resulted in greatest BM donor chimerism at 1-month (~45%) compared to CT1 (13.5%) and BMT only (0.2%) (p0.01). Flow cytometry analysis demonstrated that 6.6% of total uterine cells in the CT2+SCF mice were GFP+ BM-derived cells. Remarkably, this was about 40-fold and 80-fold greater than GFP+ BMDCs in uterus of CT1 or BMT only mice (6.6% vs. 0.16% vs. 0.08%, respectively, p0.001). No GFP was detected in saline mice. Immunohistochemical analysis of GFP expression corroborated the flow cytometry data. In addition, BM-derived cells in the uterus of CT2+SCF mice were mostly localized to the endometrial stroma, with GFP+ cells representing 4.5% of total endometrial stromal cells. The majority of BM-derived GFP+ cells colocalized with the pan-leuokocyte CD45 surface marker (58.5%), but a substantial number of cells were CD45-negative (41.5%). Cytokeratin and CD31 staining showed that the CD45-GFP+ cells were not epithelial or endothelial, respectively, confirming their stromal identity.
Conclusions: We demonstrate that paired-dose 5-FU regimen results in efficient BM donor chimerism while maintaining ovarian function and fertility of the transplanted mice. Moreover, BMDCs were found in the uterus 1 month following BMT and were characterized as mainly leukocytes and stromal cells. The model described herein should be useful for the study of BMDCs cell trafficking to the uterus in various reproductive physiological and pathological conditions.