

IN VIVO IMAGING OF MGMT(P140K)-MEDIATED SELECTION REVEALS  
PERSISTENT BONE MARROW CELL COLONIES

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Abstract:

Lentiviral gene transfer into hematopoietic stem cells has been more attractive in the past decade as a safer gene therapy vector instead of gamma-retroviral vector due to low chance of insertional mutagenesis. However low transduction efficiency is an obstacle to achieve successful gene therapy. Our lab has been using mutant form of DNA repair protein, O6-methylguanine-DNA methyltransferase (MGMT-P140K), to enrich and enhance hematopoietic stem cell after transplantation in vivo. In vivo enrichment of hematopoietic stem cells was visualized with bioluminescence imaging up to 9 weeks after bone marrow transplantation. BLI showed a dynamic engraftment patterns, and after 30mg/kg Benzylguanine (BG) and 60mg/kg Temozolomide (TMZ) treatments or 7mg/kg BCNU treatment, bioluminescence imaging data showed enhanced engraftment from transplanted bone marrow cells in hematopoietic organs, such as spleen and bone marrows, as well as some persistent foci outside of common hematopoietic organs. Clonal analysis with LAM-PCR from lentiviral transduced bone marrow cells before infusion showed a great heterogeneity with different insertional profiles, however analysis of colonies from drug treated mice showed dramatic decrease in heterogeneity in various hematopoietic organs. A similar clonal profile with LAM-PCR in various organs after drug selection showed us a universal clonal selection within the animal. To further investigate those persistent foci which were hard to retrieved with BLI only, we generated lentiviral vector with both firefly luciferase and eGFP, which allowed us to use Case cryo-imaging system to accurately locate transduced bone marrow cells in mice with single cell sensitivity. In addition, cryo-imaging system made it feasible for us to retrieve GFP positive colonies for LAM-PCR analysis to further understand the clonal selection process.