ENGINEERING AN ECTOPIC NICHE FOR HEMATOPOIETIC STEM CELLS VIA ENDOCHONDRAL OSSIFICATION

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Abstract:

The bone marrow (BM) niche is commonly defined as a complex microenvironment that controls the fate of Hematopoietic Stem Cells (HSC). During embryonic development, the functional establishment of the BM niche critically relies on the process of endochondral ossification, namely the remodeling of hypertrophic cartilage into bone tissue. We thus hypothesized that tissues engineered by instructing human adult Mesenchymal Stem/Stromal Cells (hMSC) towards the endochondral route allow to generate a niche for functional HSC. hMSC were seeded on a collagen scaffold and cultured under conditions leading to terminal chondrogenic differentiation, associated with hypertrophy and mineralization. Upon implantation into immunodeficient mice, the cartilaginous template underwent extensive remodeling including vascularization by host-derived vessels (CD31+), tissue resorption mediated by osteoclasts activity (TRAP+, MMP9+), and formation of mineralized bony tissue associated with BM thus defining an Ectopic Ossicle (EO). FACS analysis of the EO-derived BM displayed similar frequencies of different cell types as compared to control BM (native femur), including Lin^{-} $Sca^{+}Kit^{+}CD34^{-}CD135^{-}CD150^{+}$ HSC.

Whole EO-derived BM ($CD45.2^{+}$) was then transplanted together with competitor BM ($CD45.1^{+}$) into lethally irradiated congenic mice ($CD45.1^{+}CD45.2^{+}$) to assess long-term repopulation capacity. Both EO-derived and control HSC engrafted into recipients developing blood chimerism. Analysis of blood cells at 1, 2, and 3.5 months showed similar relative frequencies of B, T, and myeloid cells among recipients that received EO and control BM. After 3.5 months, EO-derived HSC were detected in femurs, confirming successful engraftment. Moreover, relative frequencies of phenotypically defined progenitors (multipotent, megakaryocyte-erythroid, common myeloid /granulocyte-macrophage) confirmed that whole BM transplantion successfully reconstituted a functional and hierarchically complete hematopoietic compartment. In conclusion, we generated ectopic BM niches by evoking a ‘developmental engineering’ approach. These could serve as a model to investigate physiological and pathological mechanisms controlling, e.g., stem cells engraftment and homeostasis or the effect of malignancies on the stromal compartment. The model could also eventually improve critical aspects in HSC therapeutic applications like harvesting, expansion and delivery.