Summary and Conclusions

Adult stem cells hold many promises in regenerative medicine. The hematopoietic stem cell (HSC) is the best-characterized somatic stem cell so far, but in vitro expansion has been unsuccessful, limiting the future of therapeutic potential of these cultured cells. HSC niche plays a key role in its expansion. Niches are local tissue microenvironments that maintain and regulate stem cells. In mammals, the stem cell niche is a complex entity because of the high degree of interacting cellular components, many of them are unknown. It is believed that the basic mechanism of niche regulation is conserved from invertebrate to vertebrate. Bone and marrow are linked with HSCs, and the primitive HSCs are located proximal to the endosteal surface of the trabecular bone. Numerous studies have been carried out to understand the signaling molecules involved in self-renewal and differentiation of HSCs. These signaling molecules are believed to be involved in the maintenance of HSCs in vivo. The aim of this thesis is to determine the role of HSC niche in relation to HSC division upon transplantation, its cell cycle status and to identify a few factors that may control hematopoiesis but unrevealed in this regard.

To understand the underlying mechanism of HSC niche in the division of the donor transplanted HSC, experiments were performed using C57BL/6J mouse expressing alloantigen as donor and recipient mice (to avoid immunological rejection and to identify donor cells). Bone marrow cells were harvested from the donor mice and transplanted via tail vein into the sublethally irradiated recipient mice. Time dependent increase in donor stem cell (LSK) number was determined. These observations indicated that there was an increase in absolute donor stem cell population up to 15 day of transplantation and then the stem cell pool maintained the plateau. Competitive marrow repopulation assay confirmed the increase of stem cell number as in same transplantation dose more LSK cells was recovered in 15 days rather than in 10 day transplanted mouse. Cell cycle status of HSC was determined using Hoechst and Pyronin Y staining at different time points. It was found that the percentage of donor HSCs at G0 phase decreased when compared to the cells at G1 and S/G2M phase, indicating that they were active in cell cycle. In case of the recipient, maximum cells were in the quiescent (G0) stage. This was due to the competition between the donor and the host cells in which host cells were compromised as a result of radiation injury and/or donor cells were naturally activated due to removal.
Summary and Conclusions

from their niche. Immunohistochemical analysis further confirmed the colonization and localization of donor HSC in recipient mice.

In the second part of study, global gene expression analysis was performed in marrow stromal cells isolated at different times of transplantation. This study was conducted to identify key molecule(s) important for hematopoiesis and maintenance of the cells. Many genes were found to be up-regulated and down-regulated with respect to the control sample. Using molecular and genomic approaches two genes, Areg and SCGF were identified and their expressions were found up-regulated in the stromal cells. The products of these genes were secretory in nature. The roles of these molecules in HSC proliferation and differentiation were examined in sh-RNA-based knock-down cell lines and found to have anti-apoptotic effect of HSC.

Thus, to conclude this study, a combination of sub-lethal dose of irradiation and high dose of donor cells would be beneficial for reconstituting the bone marrow. Areg and SCGF, as identified, have shown to prevent apoptosis thus allowing cells to proliferate and differentiate in response to various growth factors. Therefore, their incorporation (singly or together) along with other known hematopoietic growth factors would be necessary to expand HSC in stroma-free culture condition. Since ex vivo expansion of HSCs has many clinical applications, incorporating Areg and SCGF would be promising. Furthermore, improved HSC expansion may pave the way for advancements in various cell based transplantations. Such expanded HSCs may also be used clinically for the therapy for specific diseases such as anaemia and thalassemia, where reduced numbers of HSCs have limited the success of gene therapy. Apart from that, various studies showed that hematopoietic microenvironment plays an active role in inducing and/or sustaining hematopoietic disease. Mice deficient for retinoic acid receptor gamma (RARγ) or retinoblastoma (Rb) suffer from microenvironment-induced myeloproliferative-like diseases (MPDs). A marked reduction in trabecular osteoblasts correlated with disease progression was accompanied by loss of HSCs in the bone marrow and increased mobilization of HSCs to extramedullary tissues (Walkley et al., 2007).

Furthermore, in both hematopoietic and endothelial cells conditional loss of glycoprotein 130 (gp130), resulted in hematopoietic disease, with most mice dying by 12 months of age (Yao et al., 2005). This disease attributed to lack of gp130 receptor in the endothelial cells within the BM microenvironment and not the intrinsic HSCs (Yao et al., 2005).
Chronic MPDs patients have been reported to possess high levels of VEGF (Panteli et al., 2007). Not only endothelial cells but osteoclasts and osteoblasts also express VEGF receptors, and VEGF modulates their activities (Zeitzer and Olsen, 2005). Various studies reported that the donor-derived hematopoietic disease in allogeneic bone marrow transplant recipients (Hertenstein et al., 2005; Sala-Torra et al., 2006) occurred at a frequency of approximately 1 in 800 transplant recipients and included myelodysplastic syndrome, chronic myeloid leukemia and acute myeloid leukemia. Interestingly, long-term follow up of the patients and their donors resulted in all healthy donors, implicating the microenvironment of the patient likely contributed to the disease. Future studies identifying the roles of the HSC niche in both normal and diseased states may provide new insights into the pathophysiology and potentially, treatment opportunities for treating hematologic diseases.

Future Directions

HSC niches are physiologically dynamic domains that will continue to aid in both experimental and conceptual models of tissue maintenance, and disease. The HSC niche is responsible for regulating the HSC behaviour. The present knowledge of HSC expansion has not been evolved on the basis of systematic study on marrow niche during its regeneration. As a result the performance of culture is not at a stage that can be easily translated to meet up clinical requirements. The present study will help to understand the marrow niche, which is conducive for self renewal of HSCs. It is expected that the derived knowledge will help us to design marrow-equivalent environment for expansion of HSCs. Further, it will be possible to understand regulatory cytokine profile in the function of hematopoietic system and predict hematopoietic disease due to marrow failure. Moreover, a symbiotic relationship is present within the niche under homeostatic conditions; however, the involvement of the HSC niche in the response to tissue injury and during aging and disease progression is not well understood. Additional work in the identification of genetic factors that regulate the formation, activity, and size of stem cell niches will be necessary in order to incorporate the niche into HSC-based therapies and regenerative medicine. Furthermore, in cases where a modified niche accompanies disease progression, targeting the niche (niche ablation) could be considered an alternative, powerful therapeutic approach to accompany current drug regimes and treatments.

Summary and Conclusions
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122


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131


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