# CHAPTER I

# INTRODUCTION

## 1. Background and rationale

Hematopoietic stem cells (HSCs) are the self-renewing multipotent progenitors that have ability to differentiate into all blood cell types. By their ability to reconstitute bone marrow of lethally irradiated mice after transplantation, HSCs are divided into two distinct populations, long-term HSCs (LT-HSCs) and short-term HSCs (ST-HSCs). LT-HSCs can sustain their properties, self-renewal and differentiation, throughout life and after serial transplantation whereas ST-HSCs are limited in their self-renewal activity. Therefore, the aim of HSCs research is to purify homogeneous population of LT-HSCs and maintain their properties *ex vivo*. Several methods have been established to identify HSCs. Based on immunophenotypical analysis, lineage-negative $(Lin)^-$ stem-cell antigen $1(SCA1)^+KIT^+$ (LSK) markers (Morrison and Weissman 1994) are commonly used for purifying HSCs containing population. Additional markers such as side population (SP) (Goodell et al. 1996), CD34 (Osawa et al., 1996) and Flt3 (CD135) (Adolfsson et al., 2001; Yang et al., 2005), have been used for further purify highly enriched HSC populations

HSCs were shown to localize to endosteum after homing assay (Xie et al., 2009). Osteoblasts were shown to secrete factors that regulate HSC properties in vivo and in vitro (Taichman et al. 1996; Taichman and Emerson 1998). It has been proposed that, by directly contact with osteblasts, HSCs is regulated by factors providing by these cells. While there are the numbers of research studies address the role of Wnt signaling in HSC maintenance, there is still limited data on the role of BMP and its antagonists, which are known as important regulators of hematopoiesis, in osteoblastic niche. In mutant mice with conditional inactivation of BMP receptor type IA (BMPRIA), an increase in the number of HSCs was observed in correlation with an increase in the number of N-cadherin$^+$CD45$^-$ osteoblastic cells (Zhang et al., 2003). The adhesion of HSCs to

osteoblast has been thought to be mediated by N-cadherin (Zhang et al. 2003). Consistent with these data, activation of Tie2/Ang1 signaling by Ang1 produced in osteoblasts, promote HSCs quiescence and also upregulate N-cadherin expression level (Arai et al., 2004). Increasing osteoblast number correlated with the increasing HSCs number (Calvi et al., 2003; Zhang et al., 2003). These data supported the role of osteoblasts in maintaining HSCs via N-cadherin mediated cell-cell adhesion in osteoblastic niche. Results from recent studies, however, raise the question of whether N-cadherin and osteoblast are really crucial in HSCs maintenance. HSCs identified by SLAM markers do not express N-cadherin and the deletion of N-cadherin in HSCs did not effect on HSC maintenance and hematopoiesis (Kiel et al., 2009).A better understanding of how each niche molecules combined to regulate HSC function will provide fundamental knowledge for creating effective ex vivo culture system for HSC expansion for clinical uses.

In this study, we generate the new feeder system that combines osteoblasts and cell engineered to secrete noggin. We demonstrated that noggin can counter effect of osteoblastic signals that limit HSC proliferation. Importantly, HSC cultured on the new feeder system produce more primitive colonies than osteoblast alone in long term culture assay. We also test the effect of N-cadherin directly expressed in mouse marrow stromal cell (mMSC) derived osteoblasts (MOBs) on HSC properties. Overexpression of N-cadherin in mMSCs showed greater capability to maintain CFC number of culture HSCs (LSK cells). In contrast, MOBs could maintain higher CFC number than mMSCs so the overexpression of N-cadherin in these cells did not cause the different in HSCs maintenance. Nevertheless, N-cadherin overexpressed MOBs in our new feeder system showed the effect on hematopoietic cell proliferation by reducing CD $45^{+}$ number and maintaining more CFC number than control. Therefore, our data demonatrated that N-cadherin expressed in niche cells, mMSCs and MOBs, increase their ability to regulate HSCs maintemance and function. Our data implicate the role of N-cadherin in maintaining HSCs in osteoblastic niche. Lastly, we also show the effect of IFN$\alpha$ treatement on reduction of *Dickkopf-1 (Dkk1)*, a soluble inhibitor of Wnt/beta-catenin signaling, expressed in MSCs but not in osteoblast. This result provided indirect

evidence that IFN$\alpha$ also has an effect on MSCs to induce HSCs proliferation. Knowledge of signaling generated by niche cells which control HSCs properties is very useful to apply for further ex vivo expansion and gene therapies in hematological diseases.

## 2. Research Questions

1. BMP and BMP antagonist involves in maintenance of HSCs self-renewal and differentiation or not

2. N-cadherin involves in maintenance of HSCs self-renewal and differentiation or not

## 3. Objectives

1. To develop ex vivo culture system for HSCs expansion and maintenance ex vivo

2. To study the effect of N-cadherin expressed in stromall cell-derived osteoblast on HSCs properties

## 4. Hypothesis

1. Balance between BMP and BMP antagonist regulate HSCs maintenance.

2. N-cadherin has the effect on HSCs maintenance by encouraging tightly adhesion of HSCs to niche cell and involve in regulating signaling in HSCs.