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Effect of systemic heparan sulfate haploinsufficiency on steady state hematopoiesis and engraftment of hematopoietic stem cells☆

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ABSTRACT

Heparan sulfate (HS) proteoglycans on stromal and hematopoietic stem/progenitor cells (HSPC) help form the stem cell niche, co-localize molecules that direct stem cell fate, and modulate HSPC homing and retention. Inhibition of HS function mobilizes marrow HSPC. In vitro, HSPC maintenance is influenced by stromal HS structure and concentration. Because inhibition of HS activity or synthesis may be developed for HSPC transplantation, it is important to examine if systemic HS deficiency influences hematopoiesis in vivo. In a transgenic mouse model of HS haploinsufficiency, we examined endogenous hematopoiesis and engraftment of allogeneic bone marrow. Endogenous hematopoiesis was normal except gender-specific alterations in peripheral blood monocyte and platelet counts. Donor engraftment was achieved in all mice following myeloablative irradiation, but HS deficiency in the stromal microenvironment, on HSPC, or both (the 3 test conditions), was associated with a trend towards lower donor engraftment percentage in the bone marrow. Following non-myeloablative irradiation, competitive engraftment was achieved in 22% of mice in the test conditions, vs 50% of control animals ($P = 0.03$). HS deficiency did not re-direct donor engraftment from bone marrow to spleen or liver. Normal HS levels in the stromal microenvironment and HSPC are required for HSPC engraftment following non-myeloablative conditioning.

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1. Introduction

Heparan sulfate (HS) proteoglycans are composed of a core protein and complex, variably sulfated polysaccharide side chains. These diverse molecules play essential roles in embryogenesis, post-natal tissues and disease, largely by mediating binding and modulating the function of a wide variety of cytokines, morphogens, signaling pathways and matrix components [1,2]. Besides its structure, the concentration and gradient of HS in tissues regulates the binding and diffusion of diverse morphogens and cytokines, and is thus a critical determinant of its biological activity and functional effects [3–5].

Based on a series of in vitro observations, we had proposed that structurally specific stromal HS are a critical component of the hematopoietic stem/progenitor cell (HSPC) niche [6–10]. Studies reporting that competitive inhibition of HS by systemic injection of HS-mimetics (e.g. sulfated glycans) mobilizes HSPC from the bone marrow into the blood indicated that HS contribute to HSPC retention in the bone marrow in vivo [11–14]. Most recently, elegant studies by Saez et al. [15] showed that highly selective abrogation of HS synthesis in $Mx1^+$ osteolineage cells that contribute to HSPC niche formation in mice decreases adhesion of HSPC in the BM, thereby mobilizing them into the blood and spleen. Of note, the latter experimental strategy [15] would not be expected to have altered systemic HS levels, e.g. in other types of stromal cells, the microvasculature, HSPC, or the extracellular matrix. Recent studies have shown that specific HS proteoglycans are also present on HSPC, and influence HSPC localization and engraftment [16].

A mouse model of systemic HS deficiency has been developed by gene targeting of Ext1 which encodes part of the glycosyl-transferase enzyme complex essential for production of HS [17,18]. Consistent with its essential role in HS biosynthesis, Ext1 is ubiquitously expressed in all tissues in adult mice. Homozygous null embryos ($Ext^{-/-}$) lack this enzyme throughout the body, fail to gastrulate or develop mesoderm and its derivatives including the heart, and die by embryonic day 8.5. HS is undetectable in the null state. Heterozygous animals ($Ext^{+/-}$) survive into adulthood and appear to be phenotypically normal other than mild reduction in bone length. In the heterozygous state, HS concentration is approximately 40–50% of normal, consistent with haploinsufficiency.

Our previous in vitro studies showed that long-term maintenance of primitive hematopoietic progenitors is influenced by both the structure and concentration of stromal HS [7,8]. However, it is not known to what extent the global concentration of HS in the

Abbreviations: CXCL12/SDF1, C-X-C motif chemokine 12/stromal cell derived factor-1; Ext1, exostosin-1; HS, heparan sulfate; HSPC, hematopoietic stem/progenitor cells; TFPI, tissue factor pathway inhibitor; VCAM1, vascular cell adhesion molecule-1; WT, wild type.

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hematopoietic microenvironment in vivo influences endogenous hematopoiesis and successful engraftment of transplanted HSPC. Since competitive inhibition of HS activity [11–14] or selective inhibition of HS synthesis [15] may be advanced to clinical trials for induction of HSPC mobilization for transplantation, it is important to examine the effects of systemic reduction of HS levels on hematopoiesis in vivo in animal models.

We hypothesized that partial, global deficiency of HS in the hematopoietic milieu or on HSPC in vivo may influence steady state and post-transplant hematopoiesis. To test this hypothesis, we examined endogenous hematopoiesis and HSPC engraftment after lethal and sublethal irradiation in adult heterozygous ($Ext^{+/-}$; haploinsufficient for HS) mice, in comparison to wild-type (WT; $Ext^{+/+}$) littermates.

2. Methods

2.1. Mice

All animal studies were approved by the Minneapolis VA Medical Center's Institutional Animal Care and Use Committee. Male mice heterozygous for Ext1-deficiency ($Ext1^{+/-}$) [18] were a gift from Dr. Jeffrey Esko (University of California at San Diego, CA). These mice were bred with wild type female C57BL/6 J mice from Jackson Laboratory (Bar Harbor, ME). Genotyping was performed on DNA isolated from tail clips using the following primers: forward Ext1 primer 5′-gttaccaaaacattctagcggc-3′, reverse Ext1 primer 5′-cggtgttgtctctgtcacagcg-3′ and the targeting vector primer 5′-cttctttttgcttcctccg-3′. PCR conditions were 95 °C for 5 min followed by 45 cycles of denaturing at 95 °C for 55 s, annealing at 57 °C for 55 s and extending at 72 °C for 90 s.

2.2. Peripheral blood analysis

Blood was collected from untransplanted or transplanted adult mice by retro-orbital bleeding while the mice were sedated by isoflurane. Peripheral blood counts and differential counts were measured on a Hemavet 950FS analyzer.

2.3. Bone marrow transplantation

Adult female mice were either sublethally (225 cGy) or lethally (1000 cGy) irradiated 24 h prior to transplantation. Mice were kept on acidified water following irradiation and for the duration of the experiment to prevent irradiation induced mortality [19]. Bone marrow from both femurs and both tibiae from adult donor male mice was injected via the tail vein into irradiated recipient female mice.

To examine the influence of HS deficiency in the stromal microenvironment, HSPC, or both, the following cross-transplant strategy was used:

WT donor and WT recipient (control condition)
WT donor and $Ext1^{+/-}$ recipient (HS deficient stroma)
$Ext1^{+/-}$ donor and WT recipient (HS deficient HSPC)
$Ext1^{+/-}$ donor and $Ext1^{+/-}$ recipient (HS deficient stroma and HSPC).

2.4. Assessment of male donor cell engraftment

Six months after transplantation, the bone marrow from both femurs and both tibiae of the transplanted female mice was isolated. The spleen and liver were also collected, and stored at −70 °C. One million unsorted bone marrow cells were reserved for quantitative real time PCR (qPCR) analysis as described below. The remaining cells were stained with PeCy7-conjugated anti-CD3e, PerCP5.5-conjugated anti-Ly6G, APC–Cy7-conjugated anti-CD45R (B220) and PE-conjugated anti-CD45. All antibodies were from BD Biosciences. The stained cells were sorted on a BD FACSAria III flow cytometer.

2.5. Quantitative real time PCR

DNA was isolated from unsorted or sorted bone marrow cells using Qiagen's QIAamp DNA Micro Kit (Valencia, CA) and quantitated with the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies, Grand Island, NY). qPCR was performed on 5–10 ng of DNA using TaqMan Universal Master Mix II with UNG and mouse Sry primers and Taqman probe (Applied Biosystems, Foster City, CA). PCR conditions were 50 °C for 2 min, 95 °C for 10 min followed by 40 cycles of 95 °C (15 s) and 60 °C (1 min) on an ABI 7900HT Sequence Detection System. To calculate the percentage of male DNA in a sample, a standard curve was generated under the same PCR conditions using a known percentage of mouse male DNA mixed with female mouse DNA (Supplementary Fig. 1). DNA was also isolated from the spleen and liver using Qiagen's DNA Blood and Tissue kit, followed by qPCR as described.

3. Results

3.1. Steady state hematopoiesis in adult heterozygous ($Ext^{+/-}$) mice

We first evaluated endogenous, steady state hematopoiesis in adult heterozygous mice of both genders, and compared it to wild type littermates of similar ages. In the peripheral blood, adequate numbers of all cell types (RBC, WBC and their subtypes, and platelets) were present

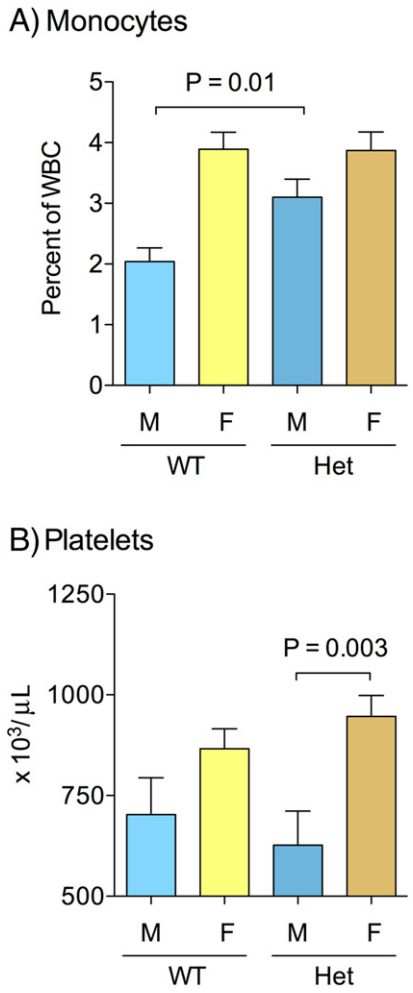

Fig. 1. Peripheral blood monocyte and platelet counts in wild type and heterozygous adult mice. Complete blood counts and WBC differential counts were determined using a Hemavet 950FS analyzer. (A) Percent monocytes, (B) platelet counts. N = 7–11 for each group. Significance of differences was determined using a 2-tailed t-test. M: male; F: female; Het: heterozygous ($Ext^{+/-}$); WT: wild type.