Inducible Sbds Deletion Impairs Bone Marrow Niche Capacity to Engraft Donor Bone Marrow After Transplantation

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Abstract:
Bone marrow (BM) niche-derived signals are critical for facilitating engraftment after hematopoietic stem cell (HSC) transplantation (HSCT). HSCT is required for restoration of hematopoiesis in patients with inherited bone marrow failure syndromes (iBMFS). Shwachman-Diamond syndrome (SDS) is a rare iBMFS associated with mutations in SBDS. Previous studies have demonstrated that SBDS deficiency in osteolineage niche cells causes bone marrow dysfunction that promotes leukemia development. However, it is unknown whether BM niche defects caused by SBDS deficiency also impair efficient engraftment of healthy donor HSC following HSCT, a hypothesis that could explain morbidity seen after clinical HSCT for patients with SDS. Here, we report a mouse model with inducible Sbds deletion in hematopoietic and osteolineage cells. Primary and secondary BM transplantation (BMT) studies demonstrated that SBDS deficiency within BM niches caused poor donor hematopoietic recovery and specifically poor HSC engraftment after myeloablative BMT. We have additionally identified multiple molecular and cellular defects within niche populations that are driven by SBDS deficiency and that are accentuated or develop specifically following myeloablative conditioning. These abnormalities include altered frequencies of multiple niche cell subsets including mesenchymal lineage cells, macrophages and endothelial cells; disruption of growth factor signaling, chemokine pathway activation, and adhesion molecule expression; and p53 pathway activation, and signals involved in cell cycle arrest. Taken together, this study demonstrates that SBDS deficiency profoundly impacts recipient hematopoietic niche function in the setting of HSCT, suggesting that novel therapeutic strategies targeting host niches could improve clinical HSCT outcomes for patients with SDS.

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Non-author contributions and disclosures: Yes; Dr. Johanna Rommens (University of Toronto) provided Sbdslox/lox mice. Dr. Peter Kurre (Children's Hospital of Philadelphia, University of Pennsylvania) made collaborative intellectual contributions to experiment design and manuscript review.

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Induction of *Sbds* deficiency in recipient bone marrow niche cells impairs efficient engraftment of healthy donor HSC after transplantation.

SBDS deficiency impairs multiple cellular signaling pathways in post-myeloablation marrow niches critical for restoration of hematopoiesis.
Abstract

Bone marrow (BM) niche-derived signals are critical for facilitating engraftment after hematopoietic stem cell (HSC) transplantation (HSCT). HSCT is required for restoration of hematopoiesis in patients with inherited bone marrow failure syndromes (iBMFS). Shwachman–Diamond syndrome (SDS) is a rare iBMFS associated with mutations in SBDS. Previous studies have demonstrated that SBDS deficiency in osteolineage niche cells causes bone marrow dysfunction that promotes leukemia development. However, it is unknown whether BM niche defects caused by SBDS deficiency also impair efficient engraftment of healthy donor HSC following HSCT, a hypothesis that could explain morbidity seen after clinical HSCT for patients with SDS. Here, we report a mouse model with inducible Sbds deletion in hematopoietic and osteolineage cells. Primary and secondary BM transplantation (BMT) studies demonstrated that SBDS deficiency within BM niches caused poor donor hematopoietic recovery and specifically poor HSC engraftment after myeloablative BMT. We have additionally identified multiple molecular and cellular defects within niche populations that are driven by SBDS deficiency and that are accentuated or develop specifically following myeloablative conditioning. These abnormalities include altered frequencies of multiple niche cell subsets including mesenchymal lineage cells, macrophages and endothelial cells; disruption of growth factor signaling, chemokine pathway activation, and adhesion molecule expression; and p53 pathway activation, and signals involved in cell cycle arrest. Taken together, this study demonstrates that SBDS deficiency profoundly impacts recipient hematopoietic niche function in the setting of HSCT, suggesting that novel therapeutic strategies targeting host niches could improve clinical HSCT outcomes for patients with SDS.
Introduction

Maintenance of hematopoietic stem cells (HSC) and downstream regulation of hematopoiesis rely upon tightly orchestrated signals generated from specialized bone marrow (BM) microenvironments known as niches. Studies over the past two decades have identified multiple BM niche cells that regulate HSC homeostasis including mesenchymal stem cells (MSC), osteolineage cells (OC), endothelial cells (EC), adipocytes, macrophages, megakaryocytes, and Schwann cells\textsuperscript{1,2}. These niche-derived signals are important not only for homeostatic hematopoiesis, but also play critical roles in facilitating engraftment after HSC transplantation (HSCT). Murine models in which selected niche components are disrupted demonstrate impaired donor HSC engraftment after HSCT\textsuperscript{3,4}.

Inherited bone marrow failure syndromes (iBMFS) are caused by germline genetic mutations in genes long known to have cell autonomous impacts on HSC and progenitor (HSPC) function. Patients with iBMFS require HSCT for cure of bone marrow failure, hematopoietic lineage deficiency, or for prevention/treatment of myelodysplastic syndrome (MDS). Recently, studies using animal models of human iBMFS have demonstrated that hematopoietic dysfunction may arise due to non-autonomous mechanisms resulting from impacts of the underlying germline mutation within BM niche cell populations\textsuperscript{5–9}. Our group has previously demonstrated that BM niche capacity to engraft healthy donor HSC after transplantation is impaired in some iBMFS, due to mutation effects within niche cells or due to pre-existence of bone marrow failure\textsuperscript{4,10}.

Shwachman–Diamond syndrome (SDS) is a rare iBMFS caused in most cases by biallelic loss-of-function mutations in genes associated with ribosome maturation including \textit{SBDS} (>90% of cases), \textit{DNAJC21}, and \textit{EFL1}, or by heterozygous mutations in \textit{SRP54}\textsuperscript{11–15}. In addition to exocrine pancreatic dysfunction, patients with SDS develop hematopoietic abnormalities including bone marrow hypocellularity, neutropenia, and variable thrombocytopenia. Approximately 20%-25% of patients will develop severe cytopenias associated with bone
marrow aplasia, and 36% of patients will develop MDS/leukemia by age 30\textsuperscript{16,17}, both requiring HSCT for cure. Unfortunately, HSCT for SDS is associated with significant rates of complications, including graft dysfunction, overt graft failure in up to 10% of patients\textsuperscript{18}, conditioning-associated organ failure, and, for patients with MDS/leukemia, high relapse risk estimated at 20%-30\%\textsuperscript{19–21}.

Skeletal dysplasia and osteopenia are also frequent manifestations of SDS\textsuperscript{22}, suggesting that bone and osteolineage cell dysfunction may be linked to hematopoietic dysfunction. Testing this hypothesis, previous studies in mouse models have shown that \textit{SBDS} deficiency in osteolineage niche cells, but not hematopoietic cells, results in bone marrow dysfunction including myelodysplasia\textsuperscript{5,6,23}. No murine models or clinical data to date address whether \textit{SBDS} deficiency causes BM niche dysfunction that contributes to poor donor engraftment outcomes after HSCT.

Herein we detail development of a mouse model defined by inducible \textit{Sbds} deletion in BM hematopoietic and osteolineage cells. Primary and secondary bone marrow transplantation (BMT) studies revealed that SBDS deficiency within BM niches results in poor hematopoietic recovery and specifically poor donor HSC engraftment after myeloablative BMT. We identified molecular and cellular abnormalities driven by SBDS deficiency within post-myeloablation niches associated with this poor engraftment phenotype. These post-BMT conditioning niche abnormalities include altered frequencies of mesenchymal lineage cells, macrophages and endothelial cells, and disruption of molecular signals in the niche including growth factor signaling, cell adhesion and homing pathways, and cell cycle/cell death pathways. Our study reveals that BMT using traditional myeloablative conditioning is associated with severe BM niche dysfunction in the setting of SBDS deficiency and identifies niche pathways altered by SBDS deficiency that are critical for efficient engraftment following BMT.

\textbf{Materials and Methods}
Animals and in vivo Treatment

Mouse colonies were maintained under pathogen-free conditions. All experiments were conducted following protocols approved by Children’s Hospital of Philadelphia (CHOP) Institutional Animal Care and Use Committee (IACUC). $Sbdsl^0$ mice (mice with 2 copies of the $Sbdsl^0$ allele) were generously provided by Dr. Johanna Rommens (University of Toronto). Other strains were obtained either from public repositories or purchased from Jackson Laboratory and backcrossed onto C57BL/6 background. $Mx1^{cre}Sbdsl^0$ mice were generated by crossing $Mx1^{cre}$ mice and $Sbdsl^0$ mice, confirming genotypes by PCR$^{24}$. To induce $Sbdsl$ deletion, polyinosinic-polycytidylic acid (pIpC, Sigma) was injected intraperitoneally (300 μg/mouse) three times per week for a minimum of two weeks. For GFP+ donor BM for BM transplantation (BMT), transgenic C57BL/6 mice expressing GFP under the H2K promoter (H2K-GFP) were used. Myeloablative total body irradiation (TBI) was performed using the X-RAD 320 (Precision X-Ray) to deliver 1100 CGy in 2 fractions, at least 3 hours apart.

Single-cell preparations from bone/BM

To prepare single BM cell suspensions, BM was flushed from leg bones, filtered (40μm mesh) and subjected to RBC lysis. To prepare single niche cell suspensions after BM flushing, leftover bones were cut into 1-2mm pieces and digested at 37˚C for 1 hour with collagenase P (Roche) or collagenase II (Gibco). Collected supernatants were filtered through 100μm mesh. Cell counts were determined using a hemocytometer and Trypan Blue (Corning).

qPCR

To measure expression of $Sbdsl$ transcripts, RNA was isolated from single cell suspensions using RNeasy Mini Kit (Qiagen). First-strand cDNA was synthesized by SuperScript™ III reverse transcription (Thermo Fisher Scientific). Quantitative PCR (qPCR) was performed on
7500 Fast Real-Time PCR System (Applied Biosystems) with SYBR Green PCR Master Mix (Applied Biosystems). Target-specific primers are described in Supplemental Table 1.

**Flow cytometry**

Flow cytometry analysis was performed on FACSCalibur or FACSJazz (Becton Dickinson). Fluorophore-conjugated antibodies used are listed in Supplemental Table 2.

**BMT assays**

In primary BMT assays, *Mx1creSbds*+/− or *Sbds*−/− recipients received 1100 cGy TBI followed by 10⁶ GFP⁺ BM cells from H2K-GFP donors via retro-orbital injection. For competitive secondary BMT assays, one week after primary BMT, BM was collected from bilateral femora/tibiae and pooled from ≥3 primary recipients per group. Pooled primary recipient BM (Dose: 1/4th total BM volume collected from one primary recipient as detailed previously⁴,¹⁰) was injected into irradiated (1100 cGy) WT secondary recipients co-transplanted with GFP⁻⁻ 2 x 10⁵ WT competitor BM cells. Secondary recipients were assessed 3-24 weeks after BMT for GFP⁺ cell reconstitution in peripheral blood lineages, including RBCs, platelets, Gr¹⁺ myeloid cells, B220⁺ B cells and CD³⁺ T cells.

**Histology and immunohistochemistry analysis**

Femora and tibiae were fixed in 10% formalin and decalcified by Regular-Cal Immuno™ (BBC Biomedicals). Samples were dehydrated before embedding in paraffin and cut into 5-6 µm sections. For general histology analysis, sections were stained with Harris hematoxylin and Eosin (Sigma-Aldrich). Immunostaining of PPAR-γ, Caspase-3, and CXCL12 were performed using primary antibodies listed in Supplemental Table 2, along with goat anti-rabbit antibody, avidin/biotin, Vector Elite ABC kit and DAB Substrate (Vector Laboratory) according to
manufacturer's instructions. Slides were examined with Zeiss AxioStar Plus and Olympus DP72 microscopic imaging.

**Multiplex ELISA**

After flushing bilateral tibiae and femora in fixed volumes of PBS (same volume for each sample to enable same dilution comparisons), samples were centrifuged at 400 x g for 5 minutes and BM plasma supernatants separated. Cell fractions were lysed in PBS containing 1% Nonidet-P40 (US Biological) and protease inhibitor (Roche). Lysates underwent 3 freeze-thaw cycles followed by centrifugation at 12000 x g for 5 minutes. BM niche cell lysates were prepared after CD45⁺ leukocyte depletion using anti-CD45 Microbeads (Miltenyi Biotec). Expression levels of 80 niche proteins were detected by Mouse Cytokine Array Q4 Kit, Mouse Cytokine Array Q5 Kit and custom-designed Quantibody Mouse Array Kits (RayBiotech).

**RNA-seq and bioinformatics analysis**

After non-adherent BM was removed by flushing, leg bones were digested by collagenase. The cell suspension from digested bones were pooled together with the BM fraction after CD45⁺ leukocyte depletion at baseline or after irradiation for RNA-seq analysis. The RNA was extracted from isolated BM and niche cell fractions using RNeasy Mini Kit (Qiagen) and DNA was removed using RNase-Free DNase Set (QIAGEN). RNA-Seq was performed by BGI Genomics (Hong Kong) on a BGISEQ-500 sequencer. Kallisto was used to perform pseudo alignment and generated read counts defined as transcripts per million (TPM)²⁵. Differential gene expression was analyzed by Deseq²⁶. Gene set enrichment analysis (GSEA) (Broad Institute) was performed using MSigDB C2 CP²⁷,²⁸ and canonical pathway gene set collection (1,027 gene sets). 1000 permutations were used to perform GSEA analysis. GSEA plots were generated to provide a graphical view of enrichment scores (ES).
Statistical Analysis

Most statistical analyses were performed using GraphPad Prism 7.00. Student’s 2-tailed t-test or Chi-squared tests were used to determine statistical significance of two-group comparisons. For RNA-seq, statistical analysis was performed using the DEseq2 package. Statistical methods for GSEA analysis were performed with GSEA software from Broad Institute, including enrichment score calculation, significance level estimation and adjustments for multiple hypothesis testing. False Discovery Rate (FDR) <0.25 and p value < 0.05 were used as significance cutoff values.

Results

SBDS deficiency within BM niche cells impairs donor engraftment after BMT

A prior mouse model targeted Sbds deletion in Osterix-expressing osteolineage progenitors. However, those mice only had a 4-week lifespan due to severe growth/development impairment, preventing that model’s use for studying niche function during HSCT. We thus attempted to target Sbds in mature osteolineage cells by crossing previously described Sbds<sup>−/−</sup> mice with Col1a1<sup>Cre+</sup> mice. However, Col1a1<sup>Cre+</sup>Sbds<sup>Exc</sup> progeny exhibited embryonic lethality (Supplemental Figure 1). We next generated a conditional Sbds deletion mouse model by crossing Sbds<sup>−/−</sup> mice with Mx1<sup>Cre+</sup> mice and inducing Cre expression in BM hematopoietic cells and Mx1-inducible osteolineage niche cells using plpC (Figure 1A). We first confirmed reduced, but not absent, Sbds gene expression in unsorted whole BM and isolated BM stromal cells from Mx1<sup>Cre+</sup>Sbds<sup>Exc</sup> mice 4 weeks after plpC initiation (Figure 1B, Supplemental Figure 2A), similar to hypomorphic expression seen in clinical SDS. Compared with controls, plpC-treated Mx1<sup>Cre+</sup>Sbds<sup>Exc</sup> mice develop significantly decreased platelet counts and an inverted myeloid/lymphoid WBC ratio in peripheral blood (Figure 1C, Supplemental Figure 2B), indicative of stress hematopoiesis. Examining BM, both lin’Sca1<sup>+</sup>cKit<sup>+</sup> (LSK) progenitors and long-term
(LT)-HSC (linSca1+ cKit+CD48-CD150+) were markedly reduced within 4 weeks of SBDS deficiency induction by pIpC in Mx1Cre SbdsExc mice (Figure 1D, E).

We next assessed whether SBDS deficiency within BM niche cell populations impacts capacity of pIpC-treated Mx1Cre SbdsExc mice to engraft healthy donor BM and specifically LT-HSC during BMT. We transplanted GFP+ wildtype donor BM into pIpC-treated Mx1Cre SbdsExc mice and Sbds+/l controls at 24 hours after 1100 cGy myeloablative TBI (Figure 2A). Most (90%) Mx1Cre SbdsExc BMT recipients died by day 9 after primary BMT, whereas all control recipients survived (Figure 2B). Inadequate engraftment appeared to be the cause of death in the Mx1Cre SbdsExc recipients, demonstrated by the persistence of BM aplasia by histology (Figure 2C) and low BM cell counts (Supplemental Figure 2C) at 1 week after BMT, compared to restored BM cellularity seen in control recipients. To test whether BM niche deficits caused by SBDS deficiency specifically impact engraftment of LT-HSC after BMT, we next performed competitive secondary BMT in which BM was harvested from Mx1Cre SbdsExc and control primary BMT recipients at 7 days after the primary BMT and transplanted with a fixed dose of competitor WT BM into WT secondary recipients. Secondary recipients of Mx1Cre SbdsExc versus littermate control primary recipient BM demonstrated decreased long-term GFP+ reconstitution of peripheral blood lineages from 6 to 24 weeks after secondary BMT, as well as decreased trilineage GFP+ BM hematopoiesis, indicating that engraftment of both hematopoietic progenitors and HSC was severely impaired in Mx1Cre SbdsExc versus littermate control primary recipients (Figure 2D, Supplemental Figure 2D).

SBDS deficiency alters BM niche cellular composition and niche cell gene expression in response to myeloablative TBI

To define mechanisms by which SBDS deficiency in BM niches impairs HSCT donor engraftment, we compared niche cell populations and niche gene expression in Mx1Cre SbdsExc...
mice vs Sbds<sup>−/−</sup> controls following myeloablative TBI (Experiment schema in Supplemental Figure 3). We first investigated MSC-derived stromal compartments that contribute to BMF and MDS in other models<sup>5,6,8</sup>, finding that BM of irradiated Mx1<sup>Cre</sup>Sbds<sup>Exc</sup> mice contains reduced osteoprogenitor cells (OPC, CD45<sup>+</sup>TER119<sup>−</sup>CD31<sup>−</sup>CD51<sup>−</sup>Sca<sup>−1</sup>) compared to controls (Figure 3A, B), while the OPC percentage was not significantly altered at baseline (Supplemental Figure 4A). Percentages of MSC (CD45<sup>−</sup>TER119<sup>−</sup>CD31<sup>−</sup>CD51<sup>−</sup>Sca<sup>−1</sup>) were similar between the two groups (data not shown). RNA-seq analysis revealed that BM stromal cells from irradiated Mx1<sup>Cre</sup>Sbds<sup>Exc</sup> mice and controls expressed similar levels of Runx2 (Figure 3C), which encodes the transcription factor that initially induces osteogenic differentiation of MSC. However, SBDS-deficient BM niche cells expressed higher levels of early osteoblast markers including Sp7 (Osterix) and Alpl (Alkaline phosphatase), as well as terminal osteoblast markers including Dmp1 and Spp1 (Osteopontin) (Figure 3C, D), suggesting that terminal osteoblast maturation and function may not be impaired by induced SBDS deficiency. ELISA confirmed increased BM osteopontin expression in irradiated Mx1<sup>Cre</sup>Sbds<sup>Exc</sup> mice (Figure 3E). These results suggest that while signals driving osteo-lineage commitment of MSC remain intact and in some cases are upregulated in SBDS-deficient niches in response to myeloablative TBI, the ability to maintain populations of immature osteolineage-committed progenitor cells after TBI is severely impaired by SBDS deficiency, either through increased terminal differentiation or impaired survival.

GSEA demonstrated that Sbds deletion in post-TBI BM niche cells caused upregulation in gene expression associated with adipogenesis, including upregulation of Pparg, Fabp4, and Adipoq (Figure 3F-H, Supplemental Table 3). Notably, GSEA analysis did not detect alteration of adipogenesis pathways in Mx1<sup>Cre</sup>Sbds<sup>Exc</sup> mice at baseline (Supplemental Figure 4B), suggesting that TBI induced a pro-adipogenic state in Mx1<sup>Cre</sup>Sbds<sup>Exc</sup> mice. Indeed, we identified increased adipocytes in BM sections of Mx1<sup>Cre</sup>Sbds<sup>Exc</sup> mice at 48 hours post-TBI, but not at baseline (Figure 3I). A majority of the adipocytes in irradiated Mx1<sup>Cre</sup>Sbds<sup>Exc</sup> BM are PPAR-γ-
positive (Supplemental Figure 4C). Given that previous studies have implicated BM adipocytes as the negative regulators of HSC engraftment\textsuperscript{31}, increased adipogenic differentiation of BM niche MSC may contribute to poor niche-mediated donor engraftment resulting from SBDS deficiency.

In contrast, endothelial cells (EC) comprised a significantly lower fraction of surviving niche cells in irradiated $\text{Mx}^1\text{Cre}Sbds^{\text{Exc}}$ versus control BM (Supplemental Figure 5A, B), notable given that sinusoidal EC regeneration mediated by VEGFR2 signaling is essential for HSC engraftment after HSCT\textsuperscript{32}. While VEGF-A protein levels in BM plasma supernatants were increased following TBI compared to baseline in both groups, $\text{Mx}^1\text{Cre}Sbds^{\text{Exc}}$ BM exhibited a trend (p=0.15) towards less TBI-driven increase than seen in control BM (Supplemental Figure 5C). GSEA analysis of RNA Seq data showed that while at baseline VEGF signaling pathway genes are markedly upregulated in the BM of $\text{Mx}^1\text{Cre}Sbds^{\text{Exc}}$ mice versus controls, BM stromal cells of irradiated $\text{Mx}^1\text{Cre}Sbds^{\text{Exc}}$ mice exhibit overall downregulation of the VEGF signaling pathway compared to controls (Supplemental Figure 5D, Supplemental Table 3). However, examining expression of individual VEGF pathway genes within niche cells after irradiation, SBDS deficiency appears to have a complex effect, increasing expression of genes such as $Kdr$, which encodes VEGFR2, while decreasing expression of other downstream mediators (Supplemental Figure 5E).

BM niche macrophages also critically regulate HSC maintenance\textsuperscript{33}. CD11b$^+$F4/80$^+$Ly6G$^-$ macrophages were significantly increased in irradiated $\text{Mx}^1\text{Cre}Sbds^{\text{Exc}}$ BM niches compared to controls, in contrast to decreased surviving granulocytes seen in $\text{Mx}^1\text{Cre}Sbds^{\text{Exc}}$ BM 24 hours post-TBI (Supplemental Figure 6A, B). RNA Seq confirmed higher expression of niche macrophage markers including $\text{Vcam-1}$, $\text{Slglec1}$ (CD169) and $\text{Ackr1}$ (CD234)\textsuperscript{33} in irradiated $\text{Mx}^1\text{Cre}Sbds^{\text{Exc}}$ versus control niche cell populations (Supplemental Figure 6C). Interestingly, despite increased expression of genes involved in phagocytic pathways at baseline and
increased frequency of HSC niche macrophages post-TBI in SBDS-deficient mice, GSEA of RNA-seq revealed significantly downregulated expression of FcγR-mediated phagocytosis pathway genes in irradiated Mx1CreSbdsExc BM niches (Supplemental Figure 6D, Supplemental Table 3) compared to controls, suggesting SBDS deficiency alters functions of niche macrophages following TBI.

**SBDS Deficiency within BM niche cells diminishes gene expression downstream of IGF-1 signaling.**

Our previous studies demonstrated that BM niche IGF-1R signaling is essential for osteolineage niche cell expansion post-TBI and efficient donor HSC engraftment34. IGF-1 /IGF-2 signaling pathways in the BM microenvironment also regulates homeostatic HSC function35,36, as prior studies have shown that IGFBP-2 and IGFBP-3 support HSC survival and inhibit OPC differentiation37–39. Compared to controls, BM plasma supernatants from Mx1CreSbdsExc mice contained higher levels of IGF-1 at baseline, but decreased levels of IGF-1 following TBI (Figure 4A). Protein levels of IGFBP-2 and IGFBP-3 were similar in Mx1CreSbdsExc mice versus controls at baseline, but Mx1CreSbdsExc BM exhibited increased levels of IGFBP-2 and IGFBP-3 following TBI (Figure 4A). While baseline mRNA expression in IGF-1 pathway genes was similar with or without SBDS deficiency (Supplemental Figure 7), RNA-seq analysis, GSEA and qPCR of BM niche cells from irradiated Mx1CreSbdsExc versus control mice demonstrated upregulated mRNA expression of upstream IGF-1 pathway factors Igf1, Igfbp2, and Igfbp3, but downregulated expression of downstream IGF-1 pathway signaling factors (Figure 4B-D). This downregulation of downstream IGF-1 signaling genes following TBI suggests that SBDS deficiency may impair this survival signal critical for OPC niche functions after HSCT.
BM niche SBDS deficiency during myeloablative TBI induces chemokine and adhesion molecule expression that favors inflammatory cell homing and impairs HSPC trafficking to BM.

Chemokines and adhesion molecules play critical roles in donor HSC homing and retention within BM niches following HSCT. Hypothesizing that engraftment deficits in Mx1CreSbdsExc mice may be due to impaired chemokine and adhesion molecule pathways, we tested the effect of SBDS deficiency on 20 BM chemokines levels prior to and after TBI using multiplex ELISA (Figure 5A, B, and Supplemental Table 4). While baseline chemokine levels were not altered by SBDS deficiency, 48 hours after irradiation, Mx1CreSbdsExc versus control BM demonstrated markedly reduced levels of four chemokines including CXCL1, CXCL9, CXCL12 and CCL22, and elevated expression of three pro-inflammatory chemokines CXCL13, CCL3 and CCL9 (Figure 5A, B). The upregulation of pro-inflammatory chemokines, particularly CCL3, in BM of irradiated Mx1CreSbdsExc mice may drive recruitment of pro-inflammatory macrophages and promote an inflammatory state that impairs normal hematopoiesis.\textsuperscript{40–43} Notably, CXCL12 plays well-described critical roles driving engraftment of CXCR4-expressing donor HSC within BM niches after HSCT. CXCL12 is expressed by a number of cell populations within the marrow niche,\textsuperscript{2} including osteo-lineage and endothelial cells that were reduced in Mx1CreSbdsExc BM post-TBI (Figure 3, Supplemental Figure 5). We assessed localization of BM CXCL12 expression changes caused by SBDS deficiency after TBI using immunohistochemistry (Figure 5C). Control mice exhibited foci of high CXCL12 levels, colocalizing with large cells possessing the morphologic appearance of megakaryocytes, which are CXCL12-responsive niche cells that survive TBI for several days and promote engraftment after BMT.\textsuperscript{4} In contrast these clusters of high CXCL12 expression were absent in Mx1CreSbdsExc BM (Figure 5C), correlating with a trend toward reduction in surviving megakaryocytes in post-TBI Mx1CreSbdsExc BM (Figure 5D, p=0.06). Reduced CXCL1 expression in post-TBI Mx1CreSbdsExc BM may also contribute to
engraftment deficits. In a zebrafish model, CXCR1 ligands (of which mouse CXCL1 is a homologue\textsuperscript{44}) play key supportive roles promoting donor HSPC engraftment\textsuperscript{45,46}.

Endothelial expression of selectins also regulates HSC and inflammatory cell homing to BM niches\textsuperscript{2}. Following TBI, \textit{Mx1\textsuperscript{Cre}Sbds\textsuperscript{Exc}} BM niche cells exhibited lower P-selectin and higher E-selectin protein and mRNA expression versus controls (Figure 5E, F). Since P-selectin expression on BM endothelial cells is critical for constitutive HSPC homing\textsuperscript{47}, while endothelial E-selectin promotes homing of inflammatory cells and increases HSC proliferation at the expense of self-renewal\textsuperscript{48}, dysregulated selectin expression in BM niche cells caused by SBDS deficiency may synergize with alterations in chemokine expression shown in Figure 5A-B to promote inflammatory cell recruitment to BM at the expense of hematopoietic engraftment.

**BM niche cells from \textit{Mx1\textsuperscript{Cre}Sbds\textsuperscript{Exc}} mice demonstrate dysregulated ribosomal protein gene expression and P53 pathway activation following TBI**

Multiple studies have demonstrated that aberrant p53 activation caused by ribosomal protein mutations is a critical driver of lineage-specific cell dysfunction in SDS and other ribosomopathies\textsuperscript{6,49}. GSEA analysis detected an overall downregulation in expression of genes critical for ribosome biogenesis and protein translation in \textit{Mx1\textsuperscript{Cre}Sbds\textsuperscript{Exc}} versus BM niche cells after myeloablative TBI (Figure 6A), but not in unirradiated BM stromal cells (Supplemental Figure 8A). However, expression differences caused by SBDS deficiency in individual ribosome genes after irradiation were in most cases not statistically significant (Supplemental Figure 8B, C). Increased activation of p53 signaling pathways and upregulated expression of p53 target genes were seen in BM niche cells from \textit{Mx1\textsuperscript{Cre}Sbds\textsuperscript{Exc}} versus control mice following TBI (Figure 6B-D, Supplemental Table 3). In contrast, GSEA analysis did not reach statistical significance comparing overall p53 pathway expression activation in unirradiated \textit{Mx1\textsuperscript{Cre}Sbds\textsuperscript{Exc}} versus control niche cells at baseline (Supplemental Figure 8D). SBDS deficiency caused
downregulation of genes involved in G1/S transition and DNA synthesis in S-phase in the BM niche of \( Mx1^{\text{Cre}} Sbds^{\text{Exc}} \) mice both after radiation and at baseline (Supplemental Figure 8E, F). These results suggest that baseline impairment in cell cycle transition within SBDS deficient BM niche cells is exacerbated by ribosomal dysfunction-induced upregulation of p53 pathways following myeloablative conditioning. Higher percentages of apoptotic cells (Caspase-3+) were also seen in the irradiated \( Mx1^{\text{Cre}} Sbds^{\text{Exc}} \) BM niche environment 48 hours after TBI (Supplemental Figure 8G). Thus cell cycle arrest and increased apoptosis within stromal cell populations may consequently impair niche capacity to efficiently engraft HSC following BMT.

**Discussion**

While BM failure in iBMFS has long been attributed to HSPC-intrinsic impacts of causative germline gene mutations, increasing evidence suggests that impacts of these mutations on cells comprising BM microenvironmental niches also contribute to BM failure\(^6-9\). Given that SDS and other iBMFS require HSCT to cure hematologic aspects of these diseases, and that cytotoxic conditioning may exacerbate niche dysfunction caused by iBMFS-associated gene mutations, it is critical to understand pathophysiologic mechanisms by which BM niche capacity to engraft donor HSC is disrupted in iBMFS. As gene therapy approaches and novel non-genotoxic conditioning approaches are under development for iBMFS\(^50-52\), better understanding of BM niche function intrinsic to certain gene mutations will be critical to identifying optimal curative therapy approaches for iBMFS.

We developed a murine model of SDS through conditional knockout of Sbds in \( Mx1 \)-inducible hematopoietic and osteolineage niche cells to study SBDS-deficient BM niche function during HSCT. We demonstrated that SBDS-deficient BM niches possess reduced capacity to engraft healthy donor HSC after transplantation. SBDS deficiency during myeloablative TBI led to marked alterations of BM niche cell composition including increased adipocytes and
macrophages and reduced OPC and ECs. Following myeloablative TBI, SBDS deficiency in BM niche cells disrupted IGF-1 signaling needed for osteolineage cell remodeling of endosteal niches, decreased expression of chemokines and adhesion molecules known to drive HSC and progenitor cell homing to BM, and produced gene expression changes associated with ribosome dysfunction, p53 activation, and increased cell cycle arrest in niche cells. While future functional studies are needed to define relative contributions of gene expression changes in each of these pathways to niche-mediated engraftment deficits in this model, the diversity of pathways disrupted suggests that targeted correction of just one of these pathways may not be sufficient to restore donor HSC engraftment efficiency.

We utilized an inducible SDS mouse model to study BM niche deficits during HSCT because constitutive Sbds knock-out models, including the Col1a1CreSbdsExc model we initially attempted, have phenotypes too severe to allow for HSCT studies. This Mx1CreSbdsExc has limitations in recapitulating human SDS, including the lack of developmental effects of SBDS deficiency given that Cre recombinase activity was not induced until 6 weeks of age. Mx1CreSbdsExc also did not develop neutropenia as is seen in human SDS, though absence of neutropenia has also been a feature of other murine SDS models5.

The high mortality after receiving BMT in our SBDS-deficient mice may be fully explained by the graft failure phenotype we identified, but it is important to point out that we cannot exclude the possibility that alterations in other organ function after TBI could contribute to BMT-associated mortality in SBDS-deficient recipients53. While reduced intensity conditioning, and not myeloablative TBI, is typically used as conditioning for HSCT in patients with SDS18, we chose myeloablative TBI as HSCT conditioning to define the maximal impact of conditioning on BM niche dysfunction in the setting of SBDS deficiency. Deficits seen in this study will serve as a benchmark for planned future studies to compare relative toxicities to BM niches induced by less intensive conditioning approaches including non-TBI based approaches.
Due to low survival rates of $Mx^\text{Cre}Sbds^\text{Exc}$ recipients beyond one week post-BMT, we could not determine whether poor niche function would result in permanent graft failure or delayed engraftment were the mice to survive the short term consequences of prolonged aplasia including severe anemia, bleeding, and infections. While rates of graft failure are significant in clinical HSCT for SDS, most patients ultimately do engraft, suggesting that if similar niche dysfunction exists in human SDS, supportive care enables survival until engraftment ultimately occurs.

Previous studies demonstrated that Mx1-induced cells in BM stroma are restricted to osteolineage progenitor cells$^{29}$. Therefore, targeting $Sbds$ deletion using Mx1-Cre impacts niche function through cell autonomous function of osteolineage progenitors and non-autonomous effects on other niche cells. Our data indicate that $Mx^\text{Cre}Sbds^\text{Exc}$ niches contain decreased OPCs following TBI, though interestingly increased expression of genes required for terminal OPC differentiation. While terminally differentiated osteoblasts may negatively regulate HSC pool size through production of osteopontin$^{47,54}$, immature OPC’s produce CXCL12 and other factors that promote HSC engraftment. The decrease in osteolineage cells following TBI caused by SBDS deficiency correlated with expansion of adipocytes known to inhibit efficient HSC engraftment$^{31}$, reduction in BM EC that may also impair HSC engraftment$^{32}$, and increased macrophages likely recruited through BM upregulation of proinflammatory chemokines (CCL3) and adhesion molecules (E-selectin) that may reduce survival and self-renewal of donor HSC$^{48}$.

Finally, similar to the pathogenesis of BM failure in SDS$^{5}$, we found that SBDS deficiency in BM niche cells following TBI led to increased p53 activation and cell cycle arrest gene expression. Downregulation of ribosomal protein expression caused by SBDS deficiency in Mx1-inducible OPCs was the likely trigger of p53 pathway upregulation. Ribosomal dysfunction caused by
SBDS deficiency may also impair poor engraftment through reduced protein expression of niche factors such as CXCL12 due to broadly impaired protein translation.

Taken together, our study demonstrates that the cell autonomous defects induced by SBDS-deficiency within osteolineage niche cells disrupt multiple cellular and molecular elements in the BM microenvironment (Figure 7), ultimately leading to reduced BM niche capacity to engraft donor HSC during HSCT. Further studies are needed to define the relative contribution of these multiple affected pathways toward impaired donor engraftment and to develop therapeutic strategies targeting these pathways to improve engraftment outcomes.
Data Sharing Statement

The project, including raw sequencing data, is registered to BioProject under accession number PRJNA763892. For other original data, please contact olsont@chop.edu.

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Authorship contributions

J.Z. and T.S.O. conceived of these studies, oversaw and personally performed the experiments, analyzed data, and wrote the manuscript. L.K. oversaw animal husbandry and assisted in the conduct of experiments. Y.S., X.Q., and J-M. F. provided critical technical assistance in the conduct of experiments. H-M. X. and B. E. helped design and performed analyses for RNA Seq expression data. D.V.B. critically reviewed studies in progress, contributed to manuscript editing, and provided valuable intellectual input to troubleshooting experiments and experimental design.

Conflicts of Interest
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Figure Legends

Figure 1: Mice with inducible SBDS deficiency in hematopoietic and osteolineage niche cells develop disrupted hematopoiesis consistent with BM failure. (A) Mx1\textsuperscript{Cre}\textsuperscript{+} Sbds\textsuperscript{Exc} mice were generated by crossing Mx1\textsuperscript{Cre+} mice with Sbds\textsuperscript{+/+} mice to generate Mx1\textsuperscript{Cre+} Sbds\textsuperscript{+/+} mice. Cre expression and Sbds deletion were induced in hematopoietic and Mx1-inducible niche cells by plpC treatment to create Mx1\textsuperscript{Cre+} Sbds\textsuperscript{Exc} mice. (B) After 4 weeks of plpC treatment, qPCR demonstrated reduced Sbds mRNA expression in flushed BM cells compared to plpC-treated Sbds\textsuperscript{+/+} controls. (C) Compared to plpC-treated control Sbds\textsuperscript{+/+} mice (n=7), Mx1\textsuperscript{Cre} Sbds\textsuperscript{Exc} mice (n=12) developed reduced platelet counts and an increased peripheral blood myeloid/lymphoid (M/L) cell ratio, consistent with stress hematopoiesis. (D) Representative dot plots showing decreased percentages of LSK (Lin\textsuperscript{-}cKit\textsuperscript{+}Sca-1\textsuperscript{+}) cells and CD48\textsuperscript{-}CD150\textsuperscript{+} long term HSC (LT-HSC) in total BM of Mx1\textsuperscript{Cre} Sbds\textsuperscript{Exc} versus control mice after 4 weeks of plpC treatment. (E) Mx1\textsuperscript{Cre} Sbds\textsuperscript{Exc} BM (n=2) shows severe reduction in percentages of LSK and LT-HSC compared to control BM (n=4). *p<0.05, ***p<0.001, Student’s t-test.

Figure 2: The BM niche of Mx1\textsuperscript{Cre} Sbds\textsuperscript{Exc} mice exhibits impaired capacity to engraft donor HSC after bone marrow transplantation (BMT). (A) Schematic of studies. Mx1\textsuperscript{Cre} Sbds\textsuperscript{Exc} mice and Sbds\textsuperscript{+/+} controls received BMT of 10\textsuperscript{6} whole BM from GFP\textsuperscript{+} donor mice at 24 hours after receiving 1100cGy of TBI. Donor engraftment in primary BMT recipients was assessed 1 week after primary BMT using histologic analysis and competitive secondary transplantation assays, in which defined volumes (25% of BM volume collected from bilateral hindleg bones) of whole
BM from Mx1\textsuperscript{Cre} Sbds\textsuperscript{Exc} or Sbds\textsuperscript{l/l} control primary recipients were transplanted with 2x10\textsuperscript{5} WT competitor (GFP\textsuperscript{neg}) whole BM cells into irradiated WT secondary recipients. (B) Cumulative survival curves showed increased mortality among Mx1\textsuperscript{Cre} Sbds\textsuperscript{Exc} recipients (n=10) following BMT compared with controls (n=8). ***p<0.001, Log-rank test. (C) Impaired donor engraftment was seen in Mx1\textsuperscript{Cre} Sbds\textsuperscript{Exc} primary recipients by H&E staining at 1 week post-BMT. Scale bar: 200\textmu m. (D) In competitive secondary BMT studies, donor engraftment efficiency in Mx1\textsuperscript{Cre} Sbds\textsuperscript{Exc} or control (Sbds\textsuperscript{l/l}) primary recipients (n=4 mice per group) was assessed by competitive secondary BMT assay. HSC and hematopoietic progenitor engraftment in primary recipient Mx1\textsuperscript{Cre} Sbds\textsuperscript{Exc} mice was significantly impaired, as indicated by lower GFP\textsuperscript{+} reconstitution of secondary recipients (n=14 mice per group) receiving Mx1\textsuperscript{Cre} Sbds\textsuperscript{Exc} versus control primary recipient BM in all blood lineages, including red blood cells (RBC), platelets, Gr1\textsuperscript{+} myeloid cells, B220\textsuperscript{+} B cells, CD3\textsuperscript{+} T cells and total white blood cells (WBC). *p<0.05, **p<0.01, ***p<0.001, Chi-squared test.

Figure 3: SBDS deficiency in BM niche cells results in decreased osteoprogenitor cells (OPC) and increased adipocytes in BM following TBI. (A) Representative dot plots show gating strategies used to define CD31\textsuperscript{-}CD51\textsuperscript{-}Sca-1\textsuperscript{-} osteoprogenitor cells (OPC) in CD45\textsuperscript{-}TER119\textsuperscript{-} BM stromal cells. (B) Mx1\textsuperscript{Cre} Sbds\textsuperscript{Exc} mice (n=5) show reduced percentages of OPC in CD45\textsuperscript{-}TER119\textsuperscript{-} BM stromal cells compared to controls (n=6) at 24 hours after 1100cGy TBI. (C) RNA-seq analysis demonstrating changes of mRNA expression in genes critical for osteoblast differentiation in BM stromal cells of irradiated (24 hours after 1100cGy TBI) Mx1\textsuperscript{Cre} SBDS\textsuperscript{Exc} versus control mice (n=5 mice per group). A positive log-fold change in this plot indicates higher expression in Mx1\textsuperscript{Cre} SBDS\textsuperscript{Exc} stromal cells. (D) qPCR confirms higher expression of the terminal osteoblast marker Spp1 (Osteopontin) in BM niche cells from irradiated (24 hours after 1100 cGy TBI) Mx1\textsuperscript{Cre} Sbds\textsuperscript{Exc} mice (n=3) compared to controls (n=4). (E) ELISA demonstrating
increased osteopontin expression in BM plasma supernatants harvested from $Mx1^{Cre} SBDS^{Exc}$ versus control mice 48 hours after 1100cGy TBI (n=5 for $Mx1^{Cre} SBDS^{Exc}$ group and n=6 for control group), but similar expression in the two groups at baseline (n=5 for $Mx1^{Cre} SBDS^{Exc}$ group and n=7 for control group). (F) Gene set enrichment analysis (GSEA) plot shows upregulation of adipogenesis-related gene expression in the BM stromal cells of irradiated (24 hours after 1100cGy TBI) $Mx1^{Cre} SBDS^{Exc}$ mice versus controls (n=5 mice per group). The green line in the GSEA plot represents the running enrichment score (ES) for the gene set within the ranked list of genes. The value at the peak of the green line is the final ES. The black bars in the middle of the GSEA plot represent where the genes in the gene set appear in the ranked list. NES: Normalized Enrichment Score. FDR: False Discovery Rate. (G) Increased mRNA expression of several genes related to adipogenesis, including $Pparg$, $Fabp4$, and $Adipoq$ in the BM stromal cells of irradiated (24 hours after 1100cGy TBI) $Mx1^{Cre} SBDS^{Exc}$ mice compared to controls. (H) qPCR confirmed that BM niche cells from irradiated $Mx1^{Cre} Sbds^{Exc}$ mice (n=3) exhibit higher expression of $Fabp4$ and $Adipoq$, which is critical for adipogenesis, compared to controls (n=4) at 24 hours after 1100cGy TBI. (I) H&E stains show increased adipocytes in BM from $Mx1^{Cre} SBDS^{Exc}$ versus control mice at 48 hours after 1100cGy TBI but not at baseline. Black arrowheads indicate adipocytes. Scale bar: 200µm. *$p<0.05$, **$p<0.01$, ***$p<0.001$, Student’s t-test or DESeq2 statistical test.

Figure 4: SBDS deficiency within BM niche cells diminishes gene expression downstream of IGF-1 signaling. (A) Protein expression of IGF-1, IGFBP-2 and IGFBP-3 in BM supernatants harvested from $Mx1^{Cre} SBDS^{Exc}$ and control mice at baseline and 48 hours after 1100 cGy TBI (n ≥5 per group). *$p<0.05$, **$p<0.01$, Student’s t-test. (B) Gene set enrichment analysis plot showing statistically significant alterations in IGF-1 signaling pathway gene expression in BM stromal cells from $Mx1^{Cre} SBDS^{Exc}$ mice versus controls 24 hours after TBI (n=5 per group). (C) Upregulation of upstream and downregulation of downstream signaling
genes within the IGF-1 pathway in the BM stromal cells of irradiated $Mx1^{Cre}SBDS^{Exc}$ mice compared to controls. *$p<0.05$, **$p<0.01$, ***$p<0.001$, DESeq2 statistical test. (D) qPCR validation confirms that BM niche cells from $Mx1^{Cre}Sbds^{Exc}$ mice (n=3) at 24 hours post 1100cGy TBI show increased expression of IGF-1 factor genes $igfbp2$ and $igfbp3$, along with decreased expression of downstream IGF-1 signaling pathway genes, including Raf1 and Fos, compared to irradiated controls (n=4). *$p<0.05$, **$p<0.01$, Student’s t-test.

Figure 5: SBDS deficiency induces altered BM niche expression of chemokines and selectins following TBI that favor inflammatory cell recruitment. (A) CXCL1, CXCL9, CXCL12 and CCL22 expression by ELISA, demonstrating reductions in all 4 chemokines in BM plasma supernatants of $Mx1^{Cre}Sbds^{Exc}$ (n=5) versus control mice (n=6) 48 hours after 1100 cGy TBI. (B) CXCL13, CCL3 and CCL9 expression by ELISA demonstrating increases in these pro-inflammatory chemokines in BM plasma harvested from $Mx1^{Cre}SBDS^{Exc}$ (n=5) versus controls (n=6) at 48 hours after 1100cGy TBI. (C) Immunohistochemistry staining demonstrates higher CXCL12 expression in BM from $Mx1^{Cre}Sbds^{Exc}$ mice compared to controls at 48 hours after 1100 cGy TBI. Arrowhead, CXCL12-positivity surrounds megakaryocytes. Scale bar: 500µm for the first column and 50µm for the second column. (D) Representative dot plots (top) show gating strategies used to define CD45$^+$CD41$^+$ megakaryocytes in post-TBI BM. $Mx1^{Cre}$-SBDS$^{Exc}$ mice (n=5) exhibit a trend towards decreased numbers of CD45$^+$CD41$^+$ megakaryocytes in the BM niche than controls (n=7) at 24 hours post-1100 cGy TBI (bottom). *$p=0.06$, Student’s t-test. (E) P- and E-selectin levels by ELISA in BM cell lysates and BM supernatants respectively, demonstrating lower P-selectin but higher E-selectin levels in $Mx1^{Cre}Sbds^{Exc}$ (n=5) versus control mice (n=6) 48 hours after 1100cGy TBI. (F) mRNA expression of $Selplg$ (encodes P-selectin) and $Sele$ (encodes E-selectin) in BM stromal cells of irradiated (24 hours after 1100cGy TBI) $Mx1^{Cre}SBDS^{Exc}$ mice compared to controls (n=5 mice per group). *$p<0.05$, **$p<0.01$, ***$p<0.001$. DESeq2 statistical test.
**p<0.01, ***p<0.001, Student’s t-test used for ELISA data or DESeq2 statistical test for RNA-seq data.

**Figure 6:** SBDS deficiency increases activation of p53 pathways in BM niche cells following myeloablative TBI. (A) GSEA plot showing overall downregulated expression of ribosomal proteins (left) and genes associated with peptide chain elongation (right) in BM stromal cells from irradiated (24 hours after 1100cGy TBI) Mx1\textsuperscript{Cre}SBDS\textsuperscript{Exc} mice versus irradiated controls. (B) GSEA plot shows overall increased activation of the p53 pathway in BM niches of irradiated Mx1\textsuperscript{Cre}SBDS\textsuperscript{Exc} versus control mice (n=5 mice per group). (C) Expression of individual p53 target genes is upregulated in BM niches of irradiated Mx1\textsuperscript{Cre}SBDS\textsuperscript{Exc} versus control mice (n=5 mice per group). NES: Normalized Enrichment Score. FDR: False Discovery Rate. *p<0.05, ** p<0.01, ***p<0.001, DESeq2 statistical test. (D) qPCR confirms upregulation of these p53 target genes in BM niche cells taken from irradiated Mx1\textsuperscript{Cre}SBDS\textsuperscript{Exc} (n=3) versus control mice (n=4) at 24 hours after 1100cGy TBI, *p<0.05, Student’s t-test.

**Figure 7:** Schematic of how SBDS Deficiency in the Mx1\textsuperscript{Cre}SBDS\textsuperscript{Exc} model impacts BM niche cell composition and gene expression in response to myeloablative irradiation, leading to impaired niche capacity to engraft donor HSC after HSCT. Our studies support a model by which Sbds knockdown in Mx1-inducible osteolineage niche cells impairs donor HSC engraftment after TBI. SBDS deficiency in osteoprogenitors causes ribosome dysfunction, p53 pathway activation and cell cycle arrest that impair the ability of these osteolineage cells to proliferate in response to IGF-1 and other growth factor signals after TBI and consequently, to support donor hematopoiesis. Due to cell intrinsic or extrinsic mechanisms, SBDS deficiency in niche cells also increases adipocytes and decreases endothelial cell survival in the BM microenvironment following TBI, both of which may impair donor engraftment after HSCT.
Decreased osteoprogenitor-derived CXCL12 and endothelial P-selectin expression may limit HSC homing and lodgment within BM niches after TBI. Elevation of CCL3 and endothelial E-selectin drives recruitment of inflammatory macrophages after TBI. The resulting pro-inflammatory environment may further disrupt engraftment and maintenance of donor HSPC.
Figure 1

A  $Mx1^{Cre+} \times Sbds^{+/l}$

$Mx1^{Cre+}Sbds^{wt/l} \times Sbds^{ll}$

$Mx1^{Cre+}Sbds^{ll}$

B  Bone Marrow

Sbds mRNA

Relative expression

$Sbds^{+/l}$ ctrl

$Mx1^{Cre+}Sbds^{Exc}$

C  Blood

Platelet

M/L ratio

K [μL]

Ratio

D  $Sbds^{+/l}$  $Mx1^{Cre+}Sbds^{Exc}$

Gated on Lin-

LSK 1.1%

LSK 0.23%

Gated on LSK

LT-HSC 0.037%

LT-HSC 0.015%

E

LSK

% of total BM

LT-HSC

% of total BM

- $Sbds^{+/l}$ controls
- $Mx1^{Cre+}Sbds^{Exc}$
Figure 2

A

GFP+ Donor BM
plpC → TBI:1100cGy
7 days

1st Recipients
- Mx1\text{Cre}\text{Sbsd }_{\text{Exc}}^{\text{Exc}}
- Sbsd \text{WT control}

BM → TBI:1100cGy
3-24 weeks

2nd Recipients
Analyze % GFP+ in Blood and BM cells

WT Competitor BM

B

Percent survival
Sbsd \text{WT Ctrl}
Mx1\text{Cre}\text{Sbsd}_{\text{Exc}}

Days post-BMT

C

Sbsd\text{WT Ctrl}
Mx1\text{Cre}\text{Sbsd}_{\text{Exc}}

7 days post-BMT

D

RBC
Platelets
Gr1\text{+ cells}

% Mice > 2% GFP+

B220\text{+ cells}
CD3\text{+ cells}
Total WBC

% Mice > 2% GFP+

weeks

weeks

weeks

weeks
Figure 3

A. Sbds<sup>lo</sup> and Mx<sup>1</sup>Cre Sbds<sup>Exc</sup> Gated on CD45<sup>+</sup>TER119<sup>+</sup>CD31<sup>+</sup>

B. OPC

C. Osteogenic Differentiation

D. Spp1

E. Osteopontin

F. Enrichment plot: HALLMARK_ADIPGENESIS

G. Adipogenesis

H. Relative expression

I. Baseline and 48 hours post-TBI
Figure 5

A

CXCL1

CXCL9

CXCL12

CCL22

Baseline | Post-TBI
---|---
p=0.054 | *

** | ***

pg/mL | pg/mL
300 | 5000
0 | 1000

B

CXCL13

CCL3

CCL9

Baseline | Post-TBI
---|---
pg/mL | pg/mL
400 | 1000
0 | 500

** | *

C

CXCL12

Sbds\textsuperscript{+/−}

MX\textsubscript{1\textsuperscript{Cre}}Sbds\textsuperscript{Exc}

D

Sbds\textsuperscript{+/−}

MX\textsubscript{1\textsuperscript{Cre}}Sbds\textsuperscript{Exc}

CD41

CD45

CD45\textsuperscript{−}CD41\textsuperscript{+} Megakaryocyte

E

P-selectin

E-selectin

Baseline | Post-TBI
---|---
pg/mL | pg/mL
10000 | 500
2000 | 1500

*** | *

F

Log2 Fold Change

Selplg

Sele

** | **
Figure 7

- **OB differentiation**
- **Cell arrest**
- **Osteogenesis**
- **Adipogenesis**
- **Adipocyte**
- **Marrow**
- **Bone**
- **Irradiation**
- **Osteoblast**
- **HSC**
- **Macrophage**
- **MSC**
- ** OPC**
- **SBDS**
- **p53**
- **CCL3**
- **CXCL12**
- **IGF-1**
- **E-selectin**
- **P-selectin**
- **Homing**
- **Sinusoid**
- **Endothelial cells**

Upregulated in \( \text{Mx}^1\text{Cre} Sbds^{Exc} \text{mice} \)

Downregulated in \( \text{Mx}^1\text{Cre} Sbds^{Exc} \text{mice} \)