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A Molecular Profile of the Endothelial Cell Response to Ionizing Radiation

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Abstract

Ionizing radiation exposure can cause acute radiation sickness (ARS) by damaging the hematopoietic compartment. Radiation damages quiescent hematopoietic stem cells (HSCs) and proliferating hematopoietic cells, resulting in neutropenia, thrombocytopenia and increased risk for long-term hematopoietic dysfunction and myelodysplasia. While some aspects of the hematopoietic response to radiation injury are intrinsic to hematopoietic cells, the recovery of the HSC pool and overall hematopoiesis is also dependent on signals from bone marrow endothelial cells (BM ECs) within the HSC vascular niche. The precise mechanisms through which BM ECs regulate HSC regeneration remain unclear. Characterization of the altered EC gene expression that occurs in response to radiation could provide a roadmap to the discovery of EC-derived mechanisms that regulate hematopoietic regeneration. Here, we show that 5 Gy total-body irradiation substantially alters the expression of numerous genes in BM ECs within 24 h and this molecular response largely resolves by day 14 postirradiation. Several unique and nonannotated genes, which encode secreted proteins were upregulated and downregulated in ECs in response to radiation. These results highlight the complexity of the molecular response of BM ECs to ionizing radiation and identify several candidate mechanisms that should be prioritized for functional analysis in models of hematopoietic injury and regeneration.

INTRODUCTION

Endothelial cells (ECs) contribute to distinct vascular systems throughout the body, including the lymphatic system, the microvasculature and larger vessels (1). It has been well demonstrated that ECs are heterogeneous cell populations, as evidenced by restrictive

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function in the blood-brain barrier and permissive function in the kidney glomeruli (1–3). Furthermore, ECs secrete unique paracrine factors that are critical to the maintenance and regeneration of specific organs. For example, sinusoidal ECs in the liver produce Wnt2 and hepatocyte growth factor (HGF), which promote liver regeneration after hepatectomy (1, 4), whereas bone marrow (BM) ECs secrete pleiotrophin (PTN) and epidermal growth factor (EGF), which are important for hematopoietic regeneration after total-body irradiation (TBI) (1, 5, 6).

Hematopoietic stem cells (HSCs) depend on cues from the BM microenvironment or “niche” for their long-term maintenance and regeneration after stress or injury (7–15). Within this HSC niche, BM ECs have an instructive and essential role in promoting HSC regeneration after myelo-suppressive chemotherapy or ionizing radiation exposure (15–18). Deletion of VEGFR2⁺ ECs or administration of a neutralizing anti-VE-cadherin antibody was shown to cause significant delays in hematopoietic recovery after TBI (15, 17). Similarly, EC-specific deletion of the Notch ligand, Jagged-1 or deletion of the vascular niche-derived paracrine factor, PTN, caused significant impairment of HSC regeneration after TBI (16, 19). Conversely, deletion of the pro-apoptotic factors, *Bak* and *Bax*, in Tie2⁺ ECs or systemic administration of autologous or allogeneic ECs has been shown to radioprotect mice from the fatal hematopoietic toxicity of TBI (17, 18). While it is now well established that BM ECs have a necessary and sufficient role in promoting hematopoietic regeneration, little is known regarding the precise mechanisms through which BM ECs regulate this process.

The goal of this study was to characterize the molecular response of BM ECs to ionizing radiation as a means of identifying the cellular mechanisms and paracrine factors through which BM ECs regulate hematopoietic regeneration. Here, we provide a comprehensive analysis of BM EC gene expression over time, with a particular focus on secreted gene products, and have coupled this with analysis of cytokines in the BM over time in wild-type mice after TBI. We studied C57Bl/6 mice since this is the most widely utilized strain for hematopoietic research. We compared the molecular state of nonirradiated mice with that of irradiated (5 Gy) mice at 6 and 24 h postirradiation, since we have observed profound alterations in peripheral blood cell gene expression at these early time points (20–22), and day +14, since this represents a time point of early HSC regeneration in the BM of irradiated mice (6). We utilized the 5 Gy dose for TBI since this causes evident damage to the BM vasculature and myelosuppression, but is still sublethal. The results of these studies provide a roadmap into the mechanistic response of BM ECs to radiation as well as the foundation for functional screening of candidate genes and the potential for development of novel therapeutics to promote hematopoietic regeneration.

MATERIALS AND METHODS

Mice

All animal procedures were performed in accordance with animal use protocols approved by UCLA Institutional Animal Care and Use Committee. Female C57BL/6 mice (10–12 weeks old) from the UCLA mouse colony were used for all studies described.

Histology

Mouse endothelial cell antigen (MECA)-stained images were prepared as previously described (17). Briefly, femurs were fixed overnight in 4% paraformaldehyde (Affymetrix® Inc., Santa Clara, CA), decalcified and embedded in OCT media (Sakura Finetek U.S.A. Inc., Torrance, CA). Femurs were sectioned at 8 µm and stained for hematoxylin and anti-mouse endothelial cell antibody (MECA-32; BD Biosciences, San Jose, CA). Images were acquired at 20× magnification on a Zeiss Axio Imager M2 (Carl Zeiss MicroImaging Inc., Thornwood, NY).

Apoptosis

At 0, 6 and 24 h postirradiation, Annexin V-FITC/7AAD staining was performed on isolated BM ECs with the Annexin V-FITC kit (BD Biosciences).

Radiation Studies

Mice received 5 or 7.5 Gy TBI with a Gammacell®-40 cesium-137 irradiator (Best® Theratronics Ltd., Ottawa, Canada) at a dose rate of 0.6 Gy/min. Mice were euthanized at 6 and 24 h or 14 days postirradiation. Nonirradiated mice were used as controls.

Isolation of BM ECs

Bone marrow endothelial cells were isolated in accordance with the published protocol by Poulos *et al.* (16). Intravital labeling of ECs was achieved via intravenous injection of 25 µg of anti-VE-cadherin AF-647 (BioLegend®, San Diego, CA) into wild-type C57BL/6 mice. Mice were euthanized 15 min after injection. Femurs and tibias were dissected, cleaned of adventitia, and crushed with a mortar and pestle. Crushed BM was digested for 10 min at 37°C in 2.5 mg/ml Collagenase A and 1 unit/ml Dispase II (Life Technologies, Carlsbad, CA). Cells were rinsed in ice-cold phosphate buffered saline (PBS) with 1% fetal bovine serum (FBS) (Thermo Fisher Scientific Inc., Waltham, MA) and depleted of lineage-positive cells with the Miltenyi lineage depletion kit (Miltenyi Biotec Inc., San Diego, CA). The lineage-negative fraction was stained with anti-CD45-V450 and 7AAD (both from BD Biosciences). Cells were sorted on a BD FACSAria™ Fusion (model no. 656700-10-H-3201, BD Biosciences). BM ECs were isolated via staining for the 7AAD⁻CD45⁻ VE-cadherin⁺ fraction. Cells from five mice were pooled together to comprise one biological replicate.

Microarray Analysis

RNA was isolated using the QIAGEN® RNeasy micro kit (QIAGEN, Santa Clarita, CA). Isolated RNA was then sent to the UCLA Clinical Microarray Core (CMC) for subsequent processing. The samples were amplified using the NuGEN Ovation RNA Amplification System V2 (cat. no. 3100-A01; NuGEN Technologies Inc., San Carlos, CA) and were then hybridized to Affymetrix Mouse Genome 430 2.0 Array. Affymetrix GeneChip® CEL files for the samples assayed were imported into the Affymetrix Expression Console™ software to perform gene level normalization and signal summarizations. Affymetrix Transcriptome Analysis Console (TAC) software was used to analyze the differential gene expression between the irradiation time points. The Affymetrix TAC software computed the fold changes, ANOVA and FDR *P* value across all conditions for all the probe sets for each of the

pairwise conditions. A comparison between no irradiation and 6 h postirradiation was used for the heat map analysis in Fig. 2. Genes that demonstrated a greater than tenfold change and FDR *P* value greater than 0.05 were used for the heat map analysis. Robust multi-array average (RMA) values generated in the Affymetrix Expression Console software from this gene list were imported into Partek[®] microarray data analysis software (Partek Inc., St. Louis, MO) and unsupervised hierarchical cluster was performed on the various gene lists.

Cytokine Analysis

For Luminex[®] cytokine array analysis, eight mice were irradiated with 5 Gy and subsequently analyzed at each of the following time points: 6 and 24 h, 7 and 14 days. Nonirradiated mice were used as controls. Mice were euthanized and the BM was flushed into 400 μ l Iscove's modified Eagle medium. Cells were pelleted, the BM supernatant was collected and sent to the UCLA Immune Assessment Core for cytokine analysis using a Luminex 200[™] (Luminex Inc., Austin, TX). The samples were tested on three different platforms (EMD Millipore, Billerica, MA): Mouse 32-Plex (MCYTMAG-70K-PX32), Mouse Angiogenesis Growth Factor Panel (MAGPMAG-24K), and Mouse Bone (MBNMAG-41K). Seventy distinct cytokines were assayed.

Statistical Analysis

Comparisons of the mean percentages of Annexin⁺7AAD⁺ cells and Annexin⁺7AAD⁻ cells were performed using the Student's *t* test.

RESULTS

Irradiation Disrupts the BM Vascular Niche and Induces BM EC Death

The first goal of this study was to characterize the effect of TBI on the integrity of the BM vasculature. C57BL/6 mice that received 5 Gy irradiation displayed corruption of the BM sinusoidal vascular architecture at 6 and 24 h postirradiation, but vasculogenesis and nascent vessel formation was evident in the BM by day +14 (Fig. 1A). After 7.5 Gy TBI (LD_{50/30} level), the BM vascular disruption was comparable at 6 and 24 h postirradiation, but BM vascular recovery lagged at day +14 compared to irradiated (5 mice (Fig. 1A). We also analyzed BM EC apoptosis and necrosis at identical time points after 5 Gy TBI and observed a significant increase in Annexin⁺7AAD⁻ apoptotic cells at 24 h and 14 days after 5 Gy irradiation compared to nonirradiated mice (Fig. 1B). Interestingly, Annexin⁺7AAD⁺ necrotic BM ECs were increased at 6 and 24 h after a 5 Gy dose compared to nonirradiated mice. Of note, the percentages of necrotic BM ECs were no different than nonirradiated mice at day +14 (Fig. 1B).

BM ECs Express Distinct Molecular Profiles over Time after TBI

To identify BM EC genes with expression that was significantly altered by TBI, adult C57BL/6 mice received 5 Gy irradiation and then BM CD45⁻VE-cadherin⁺ cells were collected at +6 and +24 h and +14 days postirradiation. We compared the gene expression profile of these groups with that of nonirradiated BM ECs, isolated identically. Principal component analysis demonstrated that the molecular profile of each group was distinct from that of the other three groups, suggesting a dynamic progression of gene expression changes

in BM ECs over time after TBI (Fig. 2A). Hierarchical cluster analysis of 340 genes revealed that the molecular profiles of BM ECs at 6 and 24 h post-TBI were quite similar to each other and both were strikingly different from nonirradiated BM ECs (Fig. 2B). Interestingly, the day 14 post-TBI BM EC group displayed a molecular profile that was more comparable to nonirradiated BM ECs than to either the 6 or 24 h post-TBI groups.

Examination of the specific genes that were downregulated in BM ECs in response to TBI revealed several genes, including tachykinin 2, aprataxin and PNKP-like factor (*Aplf*) that were down-modulated more than 50-fold compared to nonirradiated BM ECs (Table 1). Conversely, several genes were upregulated more than 20-fold within 6 h post-TBI, including fibroblast growth factor 23 (*Fgf23*), endothelin 1 and secreted semaphorin 3f (*Sema3f*) (Table 2). Numerous genes were found in common between the 6 and 24 h time points (Tables 3, 4 and Fig. 2C). However, only two genes, which were nonannotated, were found in common between the 6 and 24 h and 14 day groups (transcript cluster ID 1441187_at and ID 144897_at; Fig. 2C). These results suggest that the molecular response of BM ECs to ionizing radiation largely resolves within 14 days postirradiation.

BM ECs Differentially Express Genes that Encode Secreted Proteins after TBI

BM ECs have been shown to regulate HSC regeneration via secretion of paracrine factors such as PTN, EGF and Jagged-1 (5, 6, 16). We next interrogated the secretome of BM ECs after TBI, leveraging an analytical technique described by Diehn *et al.* (23). We identified numerous genes that encode secreted proteins that were downregulated or upregulated more than tenfold at 6 h after 5 Gy irradiation compared to nonirradiated BM ECs (Fig. 3, Table 5), and a large number of genes were found in common between the 6 and 24 h groups (Supplementary Table 1; <http://dx.doi.org/10.1667/RR14444.1.S1>). These results suggest that BM ECs provide complex paracrine instructions to the hematopoietic system after TBI.

In a complementary analysis, we measured levels of cytokines and chemokines in the BM of nonirradiated C57Bl6 mice and at 6 and 24 h and 14 days after 5 Gy TBI. A subset of 5 cytokines displayed correlation between the increase in gene expression in BM ECs and cytokine levels in the BM after TBI (Fig. 4). In particular, FGF23 displayed marked increase in expression at 6 h, coupled with a several-fold rise in protein levels. Sclerostin (*SOST*), PECAM and HGF also displayed parallel increases in gene expression in BM ECs and protein levels in the BM. These results suggest a functional correlation between BM EC gene expression and the levels of their secreted products in the BM after TBI.

TBI Alters the Gene Expression of BM EC-Derived Regenerative Factors

Finally, we interrogated the effect of TBI on the expression of genes whose gene products are known to regulate hematopoietic regeneration. Interestingly, TBI acutely increased the expression of PTN, a heparin binding growth factor that has been shown to promote HSC regeneration in the BM vascular niche (Table 6) (5, 19, 24). Jagged-1, a Notch ligand that has been implicated in regulating HSC regeneration after irradiation, also increased moderately after TBI, whereas EGF did not increase in this analysis. Furthermore, the levels of several noncanonical Wnt proteins, *Wnt6*, *Wnt5b* and *Wnt7b*, increased substantially in BM ECs early after TBI, whereas *Wnt8b*, a canonical Wnt factor, was downregulated in BM

ECs after TBI. Conversely, Wnt5a, a noncanonical Wnt factor, was downregulated 21.2-fold in BM ECs after TBI. These results suggest that Wnt signaling may have nuanced function in regulating hematopoietic regeneration after irradiation and the balance between canonical and noncanonical Wnt signaling in this context bears further investigation (25, 26). Lastly, the levels of Neurocan and Perlecan, 2 proteoglycans that bind protein tyrosine phosphatase-sigma, were also increased in BM ECs early after TBI. This is noteworthy since PTP sigma^{-/-} mice were recently shown to have substantially increased HSC repopulating capacity compared to PTP sigma^{+/+} mice and heparan sulfates have been shown to regulate HSC retention in the niche (27, 28). Our results suggest the importance of functional examination of the role of these proteoglycans in regulating HSC regeneration *in vivo*.

DISCUSSION

Recent studies have highlighted the essential and powerful role of the BM vascular niche in regulating hematopoietic regeneration after genotoxic stresses, including ionizing radiation (15–19, 29). In particular, it was recently discovered that BM EC-derived paracrine factors regulate hematopoietic regeneration after TBI, although much remains unknown regarding the mechanisms through which the BM microenvironment regulates this process (15–19). Here, we sought to characterize the molecular response of BM ECs in mice after 5 Gy TBI, since this dose is known to cause a decline in BM HSC and progenitor cell content, with the nadir being approximately day +10 postirradiation (6). We hypothesized that genetic changes occur in BM ECs in response to TBI that can inform our understanding of the role of BM ECs in regulating the hematopoietic response to injury, and that such changes occur within hours after TBI. Our hypothesis is supported by the observation of BM vascular damage within 6 h after 5 Gy (Fig. 1). Initial recovery of phenotypic HSCs is evident at day +14 in C57Bl/6 mice exposed to >5 Gy doses (6). As such, we postulate that gene expression changes in BM ECs through day +14 after 5 Gy irradiation may provide a roadmap to discover BM EC-derived mechanisms that promote HSC regeneration. In addition, recovery of neutropenia and thrombocytopenia commence in irradiated mice at approximately day +14 and are essential for survival after TBI. We postulate that the molecular response of BM ECs to 5 Gy at early time points and day +14 can also provide insight into the role of BM ECs in regulating the recovery of neutrophils and megakaryocytes after irradiation.

Our findings highlight the damaging effects of acute, high-dose radiation on the BM vascular compartment and are consistent with prior studies of irradiated mice performed by our laboratory and others (15–19, 29). Importantly, Kopp *et al.* (30) showed that TBI causes BM EC apoptosis and dilatation of the BM vasculature, yielding plasma leakage and hemorrhage in the marrow space. Fliedner *et al.* (31) described the deleterious effects of radiation on the BM vasculature in rodents as early as 1961 and Olivos *et al.* recently summarized the current state of knowledge in this regard (32). Also of importance is that formal analysis of the effects of acute radiation exposure on the human BM vasculature or human BM ECs has not been performed. Our results suggest that evaluation of changes in the BM vasculature or gene expression analysis of BM ECs in humans over time after TBI could be highly beneficial to the field. Of note, our findings on the effects of radiation on the BM vasculature are comparable to the effects of radiation on the vasculature in other organ

systems. Adamson *et al.* demonstrated that 6.5 Gy irradiation caused marked damage to the pulmonary vasculature within 2 weeks of exposure, and it was concluded that radiation was the inciting factor in the evolution of pulmonary fibrosis in irradiated hosts (33). Similarly, Lee *et al.* demonstrated that p53 deletion in ECs exacerbated myocardial injury after radiation injury, suggesting a critical role for ECs in regulating cardiac response to irradiation (34).

Complete characterization of the mechanisms through which BM ECs regulate hematopoietic regeneration, via cell-cell mediated and secreted signals has yet to be achieved. Prior studies have identified alterations in specific cell adhesion molecules and cytokines produced by EC lines and EPC subsets after irradiation (35–39). Here, we have examined the changes in expression of non-annotated BM EC genes as well as genes that encode proteins involved in the regenerative process. Interestingly, the expression of PTN, an HSC regenerative factor, was strongly induced in BM ECs by 5 Gy irradiation, whereas Jagged-1, a Notch ligand that was recently shown to have an important role in HSC regeneration, was more modestly induced (6). The expression of several Wnt ligands as well as modulators of PTP sigma receptor signaling was also altered in BM ECs after 5 Gy TBI, suggesting that additional study of these specific proteins is merited in the context of hematopoietic injury.

Prior to this study, the most comprehensive analysis of gene expression in ECs after injury was that by Nolan *et al.* (1). In that study, the authors identified several genes that encoded “angiocrine” factors, which were upregulated in expression at day +10 and +28 after 6.5 Gy TBI, including Jagged-1, CXCL12 and VEGF-c (1). HGF expression was found to be decreased at day +10 and +28 after 6.5 Gy (1), whereas we found HGF expression to be increased in BM ECs at 6 h and 24 h after 5 Gy, and then decreased at day +14. These results reveal a bimodal response of HGF expression in BM ECs to radiation exposure. We also identified four BM EC genes whose expression changes correlated with protein levels in the BM after 5 Gy irradiation: Fgf23, Leptin, SOST and PECAM. While none of these factors are known to promote HSC regeneration after injury, Fgf23 was previously shown to induce senescence in mesenchymal stem cells from muscle (40). Deletion of SOSTDC1, a paralog of SOST, was shown to promote periosteal mesenchymal stem cell expansion and promote fracture healing in bone (41). Conversely, Leptin expression has been shown to be increased in the injured retina and necessary for retinal regeneration (42).

Our analyses highlight several important findings. First, the molecular response of BM ECs to ionizing radiation is dynamic over time and is largely resolved by two weeks after injury. Second, numerous genes that encode secreted products are strongly upregulated and downregulated after irradiation, suggesting that BM ECs produce a repertoire of paracrine factors that may contribute to the hematopoietic regenerative process. Third, functional screening studies are necessary for determining the function of these candidate BM EC-derived gene products in regulating HSC regeneration. In addition, it is important to note that BM ECs likely produce numerous growth factors that promote hematopoietic progenitor cell expansion and such factors may profoundly impact survival after radiation injury (43).

We have reported here on new information about the differential expression of annotated and nonannotated genes in BM ECs after radiation injury, and moving forward, we will focus on defining the function of several of these novel genes in regulating normal and regenerative hematopoiesis.

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References

1. Nolan D, Ginsberg M, Israely E, Palikuqi B, Poulos M, James D, et al. Molecular signatures of tissue-specific microvascular endothelial cell heterogeneity in organ maintenance and regeneration. *Dev Cell*. 2013; 26:204–19. [PubMed: 23871589]
2. Rubin L, Staddon J. The cell biology of the blood-brain barrier. *Annu Rev Neurosci*. 1999; 22:11–28. [PubMed: 10202530]
3. Churg J, Grishman E. Ultrastructure of glomerular disease: a review. *Kidney Int*. 1975; 7:254–61. [PubMed: 806738]
4. Ding B, Nolan D, Butler J, James D, Babazadeh A, Rosenwaks Z, et al. Inductive angiocrine signals from sinusoidal endothelium are required for liver regeneration. *Nature*. 2010; 468:310–5. [PubMed: 21068842]
5. Himburg H, Muramoto G, Daher P, Meadows S, Russell J, Doan P, et al. Pleiotrophin regulates the expansion and regeneration of hematopoietic stem cells. *Nat Med*. 2010; 16:475–82. [PubMed: 20305662]
6. Doan PL, Himburg HA, Helms K, Russell JL, Fixsen E, Quarmyne M, et al. Epidermal growth factor regulates hematopoietic regeneration after radiation injury. *Nat Med*. 2013; 19:295–304. [PubMed: 23377280]
7. Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature*. 2010; 466:829–34. [PubMed: 20703299]
8. Ding L, Suanders T, Enikolopov G, Morrison S. Endothelial and perivascular cells maintain haematopoietic stem cells. *Nature*. 2012; 481:457–62. 202.
9. Ding L, Morrison SJ. Haematopoietic stem cells and early lymphoid progenitors occupy distinct bone marrow niches. *Nature*. 2013; 495:231–5. [PubMed: 23434755]
10. Greenbaum A, Hsu YM, Day RB, Schuettpelz LG, Christopher MJ, Borgerding JN, et al. CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. *Nature*. 2013; 495:227–30. [PubMed: 23434756]
11. Chow A, Lucas D, Hidalgo A, Méndez-Ferrer S, Hashimoto D, Scheiermann C, et al. Bone marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche. *J Exp Med*. 2011; 208:261–71. [PubMed: 21282381]
12. Zhao M, Perry JM, Marshall H, Venkatraman A, Qian P, He XC, et al. Megakaryocytes maintain homeostatic quiescence and promote post-injury regeneration of hematopoietic stem cells. *Nat Med*. 2014; 20:1321–6. [PubMed: 25326798]
13. Kunisaki Y, Bruns I, Scheiermann C, Ahmed J, Pinho S, Zhang D, et al. Arteriolar niches maintain haematopoietic stem cell quiescence. *Nature*. 2013; 502:637–43. [PubMed: 24107994]
14. Morrison S, Scadden D. The bone marrow niche for haematopoietic stem cells. *Nature*. 2014; 505:327–34. [PubMed: 24429631]
15. Hooper AT, Butler JM, Nolan DJ, Kranz A, Iida K, Kobayashi M, et al. Engraftment and reconstitution of hematopoiesis is dependent on VEGFR2-mediated regeneration of sinusoidal endothelial cells. *Cell Stem Cell*. 2009; 4:263–74. [PubMed: 19265665]

16. Poulos MG, Guo P, Kofler NM, Pinho S, Gutkin MC, Tikhonova A, et al. Endothelial Jagged-1 is necessary for homeostatic and regenerative hematopoiesis. *Cell Rep.* 2013; 4:1022–34. [PubMed: 24012753]
17. Salter AB, Meadows SK, Muramoto GG, Himburg H, Doan P, Daher P, et al. Endothelial progenitor cell infusion induces hematopoietic stem cell reconstitution in vivo. *Blood.* 2009; 113:2104–7. [PubMed: 19141867]
18. Doan P, Russell JL, Himburg HA, Helms K, Harris JR, Lucas J, et al. Tie2(+) bone marrow endothelial cells regulate hematopoietic stem cell regeneration following radiation injury. *Stem Cells.* 2013; 31:327–37. [PubMed: 23132593]
19. Himburg HA, Harris JR, Ito T, Daher P, Russell JL, Quarmyne M, et al. Pleiotrophin regulates the retention and self-renewal of hematopoietic stem cells in the bone marrow vascular niche. *Cell Rep.* 2012; 2:964–75. [PubMed: 23084748]
20. Dressman HK, Muramoto GG, Chao NJ, Meadows S, Marshall D, Ginsburg GS, et al. Gene expression signatures that predict radiation exposure in mice and humans. *PLoS Med.* 2007; 4:e106. [PubMed: 17407386]
21. Meadows SK, Dressman HK, Muramoto GG, Himburg H, Salter A, Wei Z, et al. Gene expression signatures of radiation response are specific, durable and accurate in mice and humans. *PLoS One.* 2008; 3:e1912. [PubMed: 18382685]
22. Meadows S, Dressman H, Daher P, Himburg H, Russell J, Doan P, et al. Diagnosis of partial body radiation exposure in mice using peripheral blood gene expression profiles. *PLoS One.* 2010; 5:e11535. [PubMed: 20634956]
23. Diehn M, Eisen M, Botstein D, Brown P. Large-scale identification of secreted and membrane-associated gene products using DNA microarrays. *Nat Genet.* 2000; 25:58–62. [PubMed: 10802657]
24. Himburg HA, Yan X, Doan PL, Quarmyne M, Micewicz E, McBride W, et al. Pleiotrophin mediates hematopoietic regeneration via activation of RAS. *J Clin Invest.* 2014; 124:4753–8. [PubMed: 25250571]
25. Lento W, Ito T, Zhao C, Harris J, Huang W, Jiang C, et al. Loss of beta catenin triggers oxidative stress and impairs hematopoietic regeneration. *Genes Dev.* 2014; 28:995–1004. [PubMed: 24788518]
26. Yoon J, Ng A, Kim B, Bianco A, Xavier R, Elledge S. Wnt signaling regulates mitochondrial physiology and insulin sensitivity. *Genes Dev.* 2010; 24:1507–18. [PubMed: 20634317]
27. Quarmyne M, Doan PL, Himburg HA, Yan X, Nakamura M, Zhao L, et al. Protein tyrosine phosphatase sigma regulates hematopoietic stem cell repopulating capacity. *J Clin Invest.* 2015; 125:177–82. [PubMed: 25415437]
28. Saez B, Ferraro F, Yusuf R, Cook C, Yu V, Pardo-Saganta A, et al. Inhibiting stromal cell heparan sulfate synthesis improves stem cell mobilization and enables engraftment without cytotoxic conditioning. *Blood.* 2014; 124:2937–47. [PubMed: 25202142]
29. Chute J, Muramoto G, Salter A, Meadows S, Rickman D, Chen B, et al. Transplantation of vascular endothelial cells mediates the hematopoietic recovery and survival of lethally irradiated mice. *Blood.* 2007; 109:2365–72. [PubMed: 17095624]
30. Kopp HG, Avecilla S, Hooper A, Rafii S. The bone marrow vascular niche: home of HSC differentiation and mobilization. *Physiology.* 2005; 20:349–56. [PubMed: 16174874]
31. Fliedner T, Bond V, Cronkite E. Structural, cytologic and autoradiographic (H3-thymidine) changes in the bone marrow following total body irradiation. *Am J Pathol.* 1961; 38:599–623. [PubMed: 13700286]
32. Olivos, D., Sellamuthu, R., Singh, P., Vemula, S., Pelus, LM., Orschell, CM., et al. Stem cell niche-radiobiological response. In: Turksen, K., editor. *Biology in stem cell niche, stem cell biology and regenerative medicine.* Cham, Switzerland: Springer International Publishing; 2015.
33. Adamson I, Bowden D. Endothelial injury and repair in radiation-induced pulmonary fibrosis. *Am J Pathol.* 1983; 112:224–30. [PubMed: 6881289]
34. Lee C, Moding E, Cuneo K, Li Y, Sullivan J, Mao L, et al. p53 functions in endothelial cells to prevent radiation-induced myocardial injury in mice. *Sci Signal.* 2012; 234:1–23.

35. Jacobsen K, Kravitz J, Kincade P, Osmond D. Adhesion receptors on bone marrow stromal cells: in vivo expression of vascular cell adhesion molecule-1 by reticular cells and sinusoidal endothelium in normal and gamma-irradiated mice. *Blood*. 1996; 87:73–82. [PubMed: 8547679]
36. Chang C, Lerman O, Thanik V, Scharf C, Greives M, Schneider R, et al. Dose-dependent effect of radiation on angiogenic and angiostatic CXC chemokine expression in human endothelial cells. *Cytokine*. 2009; 48:295–302. [PubMed: 19782578]
37. Mazo I, Quackenbush E, Lowe J, von Adrian U. Total body irradiation causes profound changes in endothelial traffic molecules for hematopoietic progenitor cell recruitment to bone marrow. *Blood*. 2002; 99:4182–91. [PubMed: 12010824]
38. Gaugler M, Squiban C, Claraz M, Schweitzer K, Weksler B, Gourmelon P, et al. Characterization of the response of human bone marrow endothelial cells to in vitro irradiation. *Br J Haematol*. 1998; 103:980–9. [PubMed: 9886309]
39. Li X, Hu Z, Jorgenson M, Wingard J, Slayton W. Bone marrow sinusoidal endothelial cells undergo nonapoptotic cell death and are replaced by proliferating sinusoidal cells in situ to maintain the vascular niche following lethal irradiation. *Exp Hematol*. 2008; 36:1143–56. [PubMed: 18718416]
40. Sato C, Iso Y, Mizukami T, Otabe K, Sasai M, Kurata M, et al. Fibroblast growth factor-23 induces cellular senescence in human mesenchymal stem cells from skeletal muscle. *Biochem Biophys Res Commun*. 2016; 470:657–62. [PubMed: 26797283]
41. Collette NM, Yee CS, Hum NR, Muruges DK, Christiansen BA, Xie L, et al. Sostdc1 deficiency accelerates fracture healing by promoting the expansion of periosteal mesenchymal stem cells. *Bone*. 2016; 88:20–30. [PubMed: 27102547]
42. Zhao X, Wan J, Powell C, Ramachandran R, Myers M, Goldman D. Leptin and IL-6 cytokines synergize to stimulate mull glia reprogramming and retina regeneration. *Cell Rep*. 2014; 9:272–84. [PubMed: 25263554]
43. Na Nakorn T, Traver D, Weissman I, Akashi K. Myeloerythroid-restricted progenitors are sufficient to confer radioprotection and provide the majority of day 8 CFU-S. *J Clin Invest*. 2002; 109:1579–85. [PubMed: 12070305]

FIG. 1.

TBI obliterates the BM vasculature and causes rapid death of BM ECs. Panel A: Representative microscopic views of femurs from nonirradiated (top) and 5 and 7 Gy TBI mice (middle and bottom rows, respectively) are shown at the time points indicated. Femurs were stained with mouse endothelial cell antigen (MECA) antibody (brown) and hematoxylin (blue). In the top panel, red arrows indicate sinusoidal BM vessels. At day +14 postirradiation, red arrows indicate nascent vessel formation in the BM (scale bar = 20 μ m). Panel B. On the left side, the scatter plot shows the percentage Annexin⁺7AAD⁻ apoptotic cells within the BM EC population over time after 5 Gy irradiation ($n = 4-6$ /group, * $P = 0.001$, ** $P < 0.001$, Student's *t* test). On the right side, percentage Annexin⁺7AAD⁺ necrotic

BM ECs are shown over time after 5 Gy irradiation ($n = 4-6/\text{group}$, $*P = 0.0005$, $**P < 0.001$, Student's t test).

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FIG. 2.

BM ECs display a unique gene expression profile over time after 5 Gy TBI. Panel A: Principal component analysis (PCA) of the gene array data at the time points shown. Panel B: Heat map shows the 340 genes differentially expressed more than tenfold at 6 h after TBI versus no irradiation. The red indicates increased expression and green indicates decreased expression. Expression of the same genes is also shown at 24 h and 14 days post-TBI. The majority of differentially expressed genes (295) were downregulated at 6 h compared to nonirradiated BM ECs. At 14 days after 5 Gy TBI, the pattern of gene expression in BM ECs returned to that of nonirradiated BM ECs. Panel C: Venn diagram shows the numbers of expressed genes in common among the BM EC groups at 6 and 24 h and 14 days postirradiation

FIG. 3.

Expression pattern in BM ECs of genes that encode secreted proteins before and after TBI. Patterns of change in gene expression of numerous genes can be observed. Red indicates increased expression and green indicates decreased expression (n = 5/group).

FIG. 4. Gene expression of BM EC genes and correlative BM protein concentrations over time after 5 Gy irradiation. Representative gene expression of BM EC genes after TBI and corresponding protein concentrations of Fgf23 (panel A), Leptin (panel B), SOST (panel C), PECAM (panel D) and HGF (panel E) are shown.

TABLE 1

Downregulated Genes in BM ECs after TBI

Fold change	Gene symbol	Description	Transcript ID
-727.3	Cpox	Coproporphyrinogen oxidase	1440747_at
-150.5	Fn3k	Fructosamine 3 kinase	1418311_at
-122.9	Tac2	Tachykinin 2	1419411_at
-116.2	1700063H04Rik	RIKEN cDNA 1700063H04 gene	1431245_at
-104.4			1446309_at
-102.8			1421749_at
-91.1	Aplf	Aprataxin and PNKP like factor	1419773_at
-79.6	Kif18a	Kinesin family member 18A	1457895_at
-78.1	Ptdss2	Phosphatidylserine synthase 2	1453164_a_at
-76.0	Ints8	Integrator complex subunit 8	1460609_at
-71.0	Sox6	SRY (sex determining region Y)-box 6	1447655_x_at
-70.0	Btnl10	Butyrophilin-like 10	1421264_at
-69.6	Tspan8	Tetraspanin 8	1420018_s_at
-68.3	Ank1	Ankyrin 1, erythroid	1452512_a_at
-63.5	AU021001	Expressed sequence AU021001	1443399_at
-63.2			1442139_at
-60.8	Slc30a10	Solute carrier family 30, member 10	1438751_at
-57.0	Rhd	Rh blood group, D antigen	1417049_at
-55.4	9430064I24Rik	RIKEN cDNA 9430064I24 gene	1442912_at
-54.1			1441628_at
-53.7	Phyhip	Phytanoyl-CoA hydroxylase interacting protein	1455463_at
-52.5	Redrum	Erythroid developmental long intergenic non-protein coding transcript	1430839_at
-52.1	Pax9	Paired box 9	1442229_at
-51.5	Acp5	Acid phosphatase 5, tartrate resistant	1431609_a_at
-51.0	Epb4.2	Erythrocyte protein band 4.2	1417337_at
-51.0	Fam132a	Family with sequence similarity 132, member A	1455264_at
-50.7			1441637_at

TABLE 2

BM EC Upregulated Genes after TBI

Fold change	Gene symbol	Description	Transcript ID
98.7	Fgf23	Fibroblast growth factor 23	1422176_at
74.3	Mlip	Muscular LMNA-interacting protein	1430806_at
52.7	Zfp365	Zinc finger protein 365	1433583_at
37.4	Eda2r	Ectodysplasin A2 receptor	1440085_at
24.6	Cdk18	Cyclin-dependent kinase 18	1449151_at
23.0	Edn1	Endothelin 1	1451924_a_at
22.9	Dnaic1	Dynein, axonemal, intermediate chain 1	1437093_at
22.8	Saa3	Serum amyloid A 3	1450826_a_at
21.1	Spic	Spi-C transcription factor (Spi-1/PU.1 related)	1449134_s_at
20.4			1443337_at
20.3			1444540_at
19.4	Plb1	Phospholipase B1	1430666_at
17.7	Inhbb	Inhibin beta-B	1426858_at
16.6	Ccl2	Chemokine (C-C motif) ligand 2	1420380_at
15.7	Apln	Apelin	1451038_at
15.6	Tnfsf4	Tumor necrosis factor (ligand) superfamily, member 4	1421744_at
15.4	Itga2	Integrin alpha 2	1450501_at
15.1	Kcnj15	Potassium inwardly-rectifying channel, subfamily J, member 15	1450185_a_at
14.6	Tnfrsf9	Tumor necrosis factor receptor superfamily, member 9	1460469_at
14.3	Fam219a	Family with sequence similarity 219, member A	1429801_at
14.3	Mamstr	MEF2 activating motif and SAP domain	1431836_x_at
14.1	Zfp711	Zinc finger protein 711	1432750_at
13.8	9030617O03Rik	RIKEN cDNA 9030617O03 gene	1424226_at
13.8	Slc22a14	Solute carrier family 22 (organic cation transporter), member 14	1440058_at
13.8	Ccl7	Chemokine (C-C motif) ligand 7	1421228_at
13.6	Rbpjl	Recombination signal binding protein for immunoglobulin kappa J	1450433_at
12.3	Dcxr	Dicarbonyl L-xylulose reductase	1419456_at
12.2			1456593_at
11.9	C77370	Expressed sequence C77370	1419969_at
11.9	Ltb4r2	Leukotriene B4 receptor 2	1450807_at
11.6	Ptn	Pleiotrophin	1416211_a_at
11.5	Lamc3	Laminin gamma 3	1451758_at
11.3	1810011H11Rik	RIKEN cDNA 1810011H11 gene	1429604_at
11.2			1445678_at

TABLE 3

Downregulated Genes in BM ECs at 6 and 24 h Postirradiation

Fold change	Gene symbol	Description	Transcript ID
-727.3	Cpox	Coproporphyrinogen oxidase	1440747_at
-150.5	Fn3k	Fructosamine 3 kinase	1418311_at
-122.9	Tac2	Tachykinin 2	1419411_at
-79.6	Kif18a	Kinesin family member 18A	1457895_at
-78.1	Ptdss2	Phosphatidylserine synthase 2	1453164_a_at
-71.0	Sox6	SRY (sex determining region Y)-box 6	1447655_x_at
-70.0	Btnl10	Butyrophilin-like 10	1421264_at
-63.5	AU021001	Expressed sequence AU021001	1443399_at
-60.8	Slc30a10	Solute carrier family 30, member 10	1438751_at
-57.0	Rhd	Rh blood group, D antigen	1417049_at
-53.7	Phyhip	Phytanoyl-CoA hydroxylase interacting protein	1455463_at
-52.5	Redrum	Redrum, erythroid developmental long intergenic	1430839_at
-52.1	Pax9	Paired box 9	1442229_at
-51.5	Acp5	Acid phosphatase 5, tartrate resistant	1431609_a_at
-51.0	Epb4.2	Erythrocyte protein band 4.2	1417337_at
-51.0	Fam132a	Family with sequence similarity 132, member A	1455264_at
-48.1	Pola2	Polymerase (DNA directed), alpha 2	1456713_at
-45.5	Apol8	Apolipoprotein L 8	1441054_at
-45.0	Fn3k	Fructosamine 3 kinase	1442436_at
-44.9	D13Ert37e	DNA segment, Chr 13, ERATO Doi 37, expressed	1447276_at
-40.7	Cdca7	Cell division cycle associated 7	1445681_at
-40.4	Rag1	Recombination activating gene 1	1450680_at
-37.7	Slc16a10	Solute carrier family 16 (monocarboxylic acid transporters)	1453675_at
-36.3	Paqr9	Progesterone and adipoQ receptor family member IX	1436169_at
-36.2	Rhag	Rhesus blood group-associated A glycoprotein	1419014_at
-34.1	D3Ert108e	DNA segment, Chr 3, ERATO Doi 108, expressed	1459424_at
-31.2	Mis18bp1	MIS18 binding protein 1	1443667_at
-30.8	Tyms	Thymidylate synthase	1427811_at

TABLE 4

Upregulated Genes in BM ECs at 6 and 24 h Postirradiation

Fold change	Gene symbol	Description	Transcript ID
52.7	Zfp365	Zinc finger protein 365	1433583_at
37.4	Eda2r	Ectodysplasin A2 receptor	1440085_at
24.6	Cdk18	Cyclin-dependent kinase 18	1449151_at
22.8	Saa3	Serum amyloid A 3	1450826_a_at
14.1	Zfp711	Zinc finger protein 711	1432750_at
13.8	Slc22a14	Solute carrier family 22	1440058_at
11.6	Ptn	Pleiotrophin	1416211_a_at

TABLE 5

BM EC-Secreted Gene Products at 6 h after TBI

Fold change	Gene symbol	Description	Transcript ID
98.7	Fgf23	Fibroblast growth factor 23	1422176_at
23.0	Edn1	Endothelin 1	1451924_a_at
17.7	Inhbb	Inhibin beta-B	1426858_at
16.6	Ccl2	Chemokine (C-C motif) ligand 2	1420380_at
13.8	Ccl7	Chemokine (C-C motif) ligand 7	1421228_at
12.1	Pla2g2a	Phospholipase A2, group IIA (platelets, synovial fluid)	1450128_at
11.6	Ptn	Pleiotrophin	1416211_a_at
10.2	Calca	Calcitonin/calcitonin-related polypeptide, alpha	1452004_at
-10.7	Slc7a11	Solute carrier family 7 (cationic amino acid transporter, y+ system)	1443536_at
-10.7	Trim2	Tripartite motif-containing 2	1441487_at
-11.9	Gypa	Glycophorin A	1423016_a_at
-12.3	Adora2b	Adenosine A2b receptor	1450214_at
-13.0	Slc4a1	Solute carrier family 4 (anion exchanger), member 1	1431743_a_at
-13.1	Chl1	Cell adhesion molecule with homology to L1CAM	1443713_at
-14.5	Hmgcr	3-hydroxy-3-methylglutaryl-coenzyme A reductase	1451766_at
-15.2	Mgst3	Microsomal glutathione S-transferase 3	1448300_at
-15.7	Paqr9	Progesterin and adipoQ receptor family member IX	1455025_at
-16.0	Lrig1	Leucine-rich repeats and immunoglobulin-like domains 1	1449893_a_at
-16.3	Galnt10	UDP-N-acetyl-alpha-D-galactosamine	1440493_at
-16.5	Arv1	ARV1 homolog (yeast)	1429161_at
-17.7	Ank2	Ankyrin 2, brain	1440042_at
-19.2	Ryk	Receptor-like tyrosine kinase	1447400_at
-20.0	Dpp4	Dipeptidylpeptidase 4	1438279_at
-25.6	Gypa	Glycophorin A	1425643_at
-33.3	Pon2	Paraoxonase 2	1425791_at
-36.3	Paqr9	Progesterin and adipoQ receptor family member IX	1436169_at
-38.1	Sdha	Succinate dehydrogenase complex, subunit A	1433293_at
-71.0	Sox6	SRY (sex determining region Y)-box 6	1447655_x_at

TABLE 6

TBI Effects on Expression of BM EC Regenerative Factors

Gene symbol	Description	Fold change relative to nonirradiated		
		6 h	24 h	14 days
Ptn	Pleiotrophin	11.6	12.4	1.5
Ncan	Neurocan	9.1	−3.0	−5.8
Wnt6	Wingless-type MMTV integration site family, member 6	6.7	5.1	1.1
Hspg2	Perlecan (heparan sulfate proteoglycan 2)	2.9	1.4	0.5
Dll4	Delta-like 4 (Drosophila)	2.2	1.1	−2.5
Jag1	Jagged-1	1.7	3.1	1.0
Wnt5b	Wingless-type MMTV integration site family, member 5B	1.5	3.4	2.5
Angpt1	Angiopoietin 1	1.3	4.2	1.4
Wnt10a	Wingless-type MMTV integration site family, member 10A	1.3	−2.6	−2.8
Wnt7b	Wingless-type MMTV integration site family, member 7B	1.2	1.2	−5.3
Gpc1	Glypican 1	1.1	2.8	1.1
Wnt8a	Wingless-type MMTV integration site family, member 8A	1.1	1.0	−3.2
Thbd	Thrombomodulin	1.1	1.0	1.0
Egf	Epidermal growth factor	1.1	1.0	1.0
CXCL12	Chemokine (C-X-C motif) ligand 12	1.0	1.0	1.0
Wnt4	Wingless-type MMTV integration site family, member 4	1.0	3.4	1.5
Wnt8b	Wingless-type MMTV integration site family, member 8B	−2.2	−5.1	1.1
Angpt2	Angiopoietin 2	−2.8	−6.4	−3.4
Wnt5a	Wingless-type MMTV integration site family, member 5A	−21.2	2.7	1.2