Hematopoietic stem cells (HSCs) are highly susceptible to ionizing radiation–mediated death via induction of ROS, DNA double-strand breaks, and apoptotic pathways. The development of therapeutics capable of mitigating ionizing radiation–induced hematopoietic toxicity could benefit both victims of acute radiation sickness and patients undergoing hematopoietic cell transplantation. Unfortunately, therapies capable of accelerating hematopoietic reconstitution following lethal radiation exposure have remained elusive. Here, we found that systemic administration of pleiotrophin (PTN), a protein that is secreted by BM-derived endothelial cells, substantially increased the survival of mice following radiation exposure and after myeloablative BM transplantation. In both models, PTN increased survival by accelerating the recovery of BM hematopoietic stem and progenitor cells in vivo. PTN treatment promoted HSC regeneration via activation of the RAS pathway in mice that expressed protein tyrosine phosphatase receptor-zeta (PTPRZ), whereas PTN treatment did not induce RAS signaling in PTPRZ-deficient mice, suggesting that PTN-mediated activation of RAS was dependent upon signaling through PTPRZ. PTN strongly inhibited HSC cycling following irradiation, whereas RAS inhibition abrogated PTN-mediated induction of HSC quiescence, blocked PTN-mediated recovery of hematopoietic stem and progenitor cells, and abolished PTN-mediated survival of irradiated mice. These studies demonstrate the therapeutic potential of PTN to improve survival after myeloablation and suggest that PTN-mediated hematopoietic regeneration occurs in a RAS-dependent manner.
Pleiotrophin mediates hematopoietic regeneration via activation of RAS

Heather A. Himburg,1 Xiao Yan,1,2 Phuong L. Doan,3 Mamle Quarmyne,1,2 Eva Micewicz,4 William McBride,4 Nelson J. Chao,3
Dennis J. Slamon,1 and John P. Chute1,3,5,6

1Division of Hematology/Oncology, UCLA, Los Angeles, California, USA. 2Department of Pharmacology and Cancer Biology and 3Division of Hematologic Malignancies/Cellular Therapy, Duke University, Durham, North Carolina, USA. 4Department of Radiation Oncology, ‘Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research, and 5Jonsson Comprehensive Cancer Center, UCLA, Los Angeles, California, USA.

Hematopoietic stem cells (HSCs) are highly susceptible to ionizing radiation–mediated death via induction of ROS, DNA double-strand breaks, and apoptotic pathways. The development of therapeutics capable of mitigating ionizing radiation–induced hematopoietic toxicity could benefit both victims of acute radiation sickness and patients undergoing hematopoietic cell transplantation. Unfortunately, therapies capable of accelerating hematopoietic reconstitution following lethal radiation exposure have remained elusive. Here, we found that systemic administration of pleiotrophin (PTN), a protein that is secreted by BM–derived endothelial cells, substantially increased the survival of mice following radiation exposure and after myeloablative BM transplantation. In both models, PTN increased survival by accelerating the recovery of BM hematopoietic stem and progenitor cells in vivo. PTN treatment promoted HSC regeneration via activation of the RAS pathway in mice that expressed protein tyrosine phosphatase receptor-zeta (PTPRZ), whereas PTN treatment did not induce RAS signaling in PTPRZ-deficient mice, suggesting that PTN-mediated activation of RAS was dependent upon signaling through PTPRZ. PTN strongly inhibited HSC cycling following irradiation, whereas RAS inhibition abrogated PTN-mediated induction of HSC quiescence, blocked PTN-mediated recovery of hematopoietic stem and progenitor cells, and abolished PTN-mediated survival of irradiated mice. These studies demonstrate the therapeutic potential of PTN to improve survival after myeloablation and suggest that PTN-mediated hematopoietic regeneration occurs in a RAS-dependent manner.

Introduction

Total body irradiation (TBI) is successfully used in the conditioning of patients for hematopoietic cell transplantation (1). Radiation causes toxicity to hematopoietic stem cells (HSCs) through the generation of ROS, induction of DNA strand breaks and apoptosis, and damage to the BM microenvironment (2–4). Despite an understanding of mechanisms through which ionizing radiation causes hematopoietic toxicity, few effective mitigators of radiation-induced hematopoietic injury have been developed (5–9). The lack of effective mitigators for acute radiation sickness (ARS) has become a public health concern, as the risk of terrorism using radiological or nuclear devices has escalated (10, 11). Elucidation of novel mechanisms through which HSCs respond to radiation and the development of therapeutics targeting such mechanisms could potentially benefit not only victims of ARS but also patients receiving TBI for hematopoietic cell transplantation.

Results and Discussion

We tested whether systemic administration of PTN could mitigate hematopoietic injury and improve the survival of lethally irradiated mice. When administered beginning +24 hours following 700 cGy TBI, 80% of PTN-treated mice survived, compared with 33% of irradiated controls (Figure 1A). Irradiated PTN-treated mice displayed increased BM cellularity; increased BM cKIT+ SCA-1+LIN– (KSL) cells, which are enriched for hematopoietic stem
and progenitor cells (HSPCs) (22); and increased colony-forming cells (CFCs) compared with irradiated controls at day +10 (Figure 1, B and C, and Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI76838DS1). These data suggested that PTN improved survival by mitigating radiation damage to HSPCs.

Figure 1. PTN treatment improves the survival of irradiated mice and hematopoietic cell transplant recipients. (A) Survival of 700 cGy-irradiated mice treated intraperitoneally with 2 or 4 μg PTN or saline administered at +24 hours and every other day through day +14 (PTN-treated groups: 12 of 15 for both; saline-treated group: 5 of 15 mice; P = 0.002 for PTN 4 μg vs. saline, P = 0.004 for PTN 2 μg vs. saline). (B) Flow cytometric analysis of BM KSL cells from irradiated mice at day +10 treated with saline or PTN. (C) BM KSL cells and CFCs per femur (n = 6, *P = 0.02, **P = 0.0003). (D) Survival of irradiated mice treated subcutaneously, beginning at +48 hours and +96 hours, with PTN or saline (PTN 48 hours: 15 of 15 mice and PTN 96 hours: 13 of 15 mice; saline: 10 of 19 mice; P = 0.002 for PTN 48 hours vs. saline, P = 0.04 for PTN 96 hours vs. saline.). (E) Survival of irradiated mice transplanted with BM cells and treated with PTN or saline (PTN, 19 of 38 mice, 50% vs. saline, 6 of 36 mice, 17%; P = 0.003). (F) CFCs per femur at day +14 following transplantation and treatment with PTN or saline (n = 6, *P = 0.005). (G) H&E images (original magnification, ×63) of femurs at day +14 from transplanted mice treated with PTN or saline.
blocked PTN-mediated regeneration of KSL cells (Figure 3, B and C). Importantly, tipifarnib treatment also blocked PTN-mediated improvement in the survival of irradiated mice (Figure 3D). Whereas 75% of PTN-treated mice (12 of 16) survived 800 cGy TBI, 28% of mice (5 of 18) treated with PTN and tipifarnib survived. These data suggested that PTN-mediated improvement in survival of irradiated mice was dependent on RAS activation.

Interestingly, PTN treatment did not alter the percentage of apoptotic BM KSL cells early after 300 cGy irradiation in vitro (Figure 3E). However, PTN treatment significantly increased the percentage of KSL cells remaining in the G<sub>0</sub> phase of cell cycle after irradiation compared with that in control cells (Figure 3, F and G). Of note, RAS inhibition blocked PTN-mediated inhibition of HSC cycling following irradiation (Figure 3, F and G). These data suggest that PTN may promote HSC regeneration after irradiation via induction of HSC quiescence and that this effect occurs in a RAS-dependent manner.

Our findings have significant implications for the treatment of ARS. Recently, novel mechanisms have been described that may be targeted to mitigate radiation injury to the hematopoietic system (5, 6, 8, 32, 33). To our knowledge, PTN represents the first therapeutic demonstrated to improve survival when administered more than 24 hours after exposure. This could be advantageous in a mass casualty radiation disaster, in which medical care may be delayed for several days. Therefore, PTN has unique therapeutic potential to improve the survival of victims of ARS. Going forward, we will generate cell-specific genetic models to discern whether the in vivo effects of PTN treatment are HSC autonomous or also reflect indirect effects on the BM microenvironment.

Our results also suggest that PTN has therapeutic potential for patients undergoing limiting dose hematopoietic cell transplantation, such as adult cord blood transplantation, which can be complicated by delayed engraftment, graft failure, and death (34). While ex vivo CB expansion is currently being tested in clinical trials to augment hematopoietic recovery (35–37), an alternative strategy would be to administer systemic therapeutics to accelerate hematopoietic reconstitution in transplant recipients. Our results suggest that systemic PTN has therapeutic potential to accelerate hematopoietic reconstitution in such a setting.

Mechanistically, our data suggest that PTN-mediated expansion of HSPCs, in steady state or following irradiation, is dependent upon RAS activation. Overexpression of oncogenic RAS in hematopoietic cells causes a myeloproliferative disorder (38), the effects of physiologic RAS activation in HSCs are less well understood. Overexpression of oncogenic H-RAS in human HSCs, coupled with pharmacologic Ras inhibition, was previously suggested to promote HSC expansion (39). We postulate that PTN

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**Figure 2. RAS signaling is necessary for PTN-mediated HSPC expansion.** (A) p-ALK expression in BM KSL cells from the represented groups (n = 3, *P = 0.003). (B) p-GRB2 expression in BM KSL cells treated with media alone (gray curve) or PTN (red curve), with mean percentage p-GRB2 levels shown (n = 3, *P < 0.0001). (C) Representative p-ERK1/2 expression in KSL cells treated with media alone (gray curve) or PTN (red curve), with mean percentage p-ERK1/2 levels shown (n = 5, *P < 0.001). (D) p-ERF expression (green) in BM KSL cells cultured with thrombopoietin, SCF, and FLT-3 ligand (TSF), with or without PTN, and scatter plot of p-ERF levels in KSL cells (horizontal bars represent means; n = 12, *P < 0.0001). Scale bar: 10 μm. (E) CFCs per input KSL cells and percentage CFU-GEMMs at day +7 of the represented cultures (n = 3, *P = 0.04, **P = 0.01, *P = 0.02, **P = 0.03). (F) CD45.1<sup>+</sup> donor cell engraftment at 8 weeks following competitive transplantation of the progeny of 10 CD34<sup>+</sup> KSL cells cultured in the conditions shown (n = 7–11 per group, *P = 0.04, **P < 0.0001).
to the hematopoietic system. Several studies have shown the lack of efficacy of cell cycle–inducing cytokines in promoting survival when administered to mice after irradiation (24, 25). However, when administered prior to TBI, these same cytokines can radioprotect, perhaps by promoting the synchronized entry of HSCs into late S phase, a radioresistant phase of the cell cycle (24, 46). Conversely, administration of a CDK4/6 inhibitor within the first +20 hours after TBI improved the survival of lethally irradiated mice (7). Our results are most consistent with these findings and those of Cheng et al. (47), who showed that cycling HSCs from \textit{p21–/–} mice displayed increased sensitivity to 5-FU chemotherapy and poor serial transplant capability compared with more quiescent \textit{p21+/+} HSCs. Our studies suggest that PTN, a BM niche–derived protein, promotes HSC quiescence early after irradiation and powerfully mitigates radiation injury to the hematopoietic system.

**Methods**

For more detailed information, see the Supplemental Methods.

**Mice.** We used PTN-deficient (\textit{Ptn}−/−) mice and PTPRZ-deficient (\textit{Ptprz}−/−) mice as previously described (23). RAS and MEK inhibitors were provided by Christopher Counter and Donita Brady (Duke University).

**Statistics.** Survival analyses were performed using the log-rank test. Data are presented as mean ± SEM throughout, and the Student’s 2-tailed \( t \) test was used for comparisons. \( P < 0.05 \) was considered significant.
Study approval. Animal procedures followed protocols approved by the Duke University and UCLA animal care committees.

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Address correspondence to: John P. Chute, Professor of Medicine, Division of Hematology/Oncology, Eli and Edythe Broad Center for Regenerative Medicine and Stem Cell Research, UCLA, 545 OHRC, 617 Charles Young Drive, Los Angeles, California 90095, USA. Phone: 310.206.3037; E-mail: jchute@mednet.ucla.edu.

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