Myeloproliferative neoplasms are clonal stem cell disorders characterized by hematopoietic stem/progenitor cell expansion. The acquired kinase mutation JAK2V617F plays a central role in these disorders. Abnormalities of the marrow microenvironment are beginning to be recognized as an important factor in the development of myeloproliferative neoplasms. Endothelial cells are an essential component of the hematopoietic vascular niche. Endothelial cells carrying the JAK2V617F mutation can be detected in patients with myeloproliferative neoplasms, suggesting that the mutant vascular niche is involved in the pathogenesis of these disorders. Here, using a transgenic mouse expressing JAK2V617F specifically in all hematopoietic cells (including hematopoietic stem/progenitor cells) and endothelial cells, we show that the JAK2V617F-mutant hematopoietic stem/progenitor cells are relatively protected by the JAK2V617F-bearing vascular niche from an otherwise lethal dose of irradiation during conditioning for stem cell transplantation. Gene expression analysis revealed that chemokine (C-X-C motif) ligand 12, epidermal growth factor, and pleiotrophin are up-regulated in irradiated JAK2V617F-bearing endothelial cells compared to wild-type cells. Our findings suggest that the mutant vascular niche may contribute to the high incidence of disease relapse in patients with myeloproliferative neoplasms following allogeneic stem cell transplantation, the only curative treatment for these disorders.

JAK2V617F-bearing vascular niche enhances malignant hematopoietic regeneration following radiation injury

Chi Hua Sarah Lin,1* Yu Zhang,2* Kenneth Kaushansky3 and Huichun Zhan1,4

1Department of Medicine, Stony Brook School of Medicine, NY, USA; 2Biopharmaceutical R&D Center, Chinese Academy of Medical Sciences & Peking Union Medical College, Suzhou, China; 3Office of the Sr. Vice President, Health Sciences, Stony Brook School of Medicine, NY, USA and 4Northport VA Medical Center, Northport, NY, USA

*CHSL and YZ contributed equally to this work.

ABSTRACT

Myeloproliferative neoplasms are clonal stem cell disorders characterized by hematopoietic stem/progenitor cell expansion. The acquired kinase mutation JAK2V617F plays a central role in these disorders. Abnormalities of the marrow microenvironment are beginning to be recognized as an important factor in the development of myeloproliferative neoplasms. Endothelial cells are an essential component of the hematopoietic vascular niche. Endothelial cells carrying the JAK2V617F mutation can be detected in patients with myeloproliferative neoplasms, suggesting that the mutant vascular niche is involved in the pathogenesis of these disorders. Here, using a transgenic mouse expressing JAK2V617F specifically in all hematopoietic cells (including hematopoietic stem/progenitor cells) and endothelial cells, we show that the JAK2V617F-mutant hematopoietic stem/progenitor cells are relatively protected by the JAK2V617F-bearing vascular niche from an otherwise lethal dose of irradiation during conditioning for stem cell transplantation. Gene expression analysis revealed that chemokine (C-X-C motif) ligand 12, epidermal growth factor, and pleiotrophin are up-regulated in irradiated JAK2V617F-bearing endothelial cells compared to wild-type cells. Our findings suggest that the mutant vascular niche may contribute to the high incidence of disease relapse in patients with myeloproliferative neoplasms following allogeneic stem cell transplantation, the only curative treatment for these disorders.

Introduction

The chronic Philadelphia chromosome (Ph1) negative myeloproliferative neoplasms (MPNs) are clonal stem cell disorders characterized by hematopoietic stem/progenitor cell (HSPC) expansion and overproduction of mature blood cells. The acquired kinase mutation JAK2V617F plays a central role in MPNs. However, the mechanisms responsible for the malignant HSPC expansion in MPNs are not fully understood, limiting the effectiveness of current treatment. Although the etiology of dysregulated hematopoiesis has been mainly attributed to the molecular alterations within the HSPC compartment, abnormalities of the marrow microenvironment are beginning to be recognized as an important factor in the development of MPNs.1-5 The diseased niche could impair normal hematopoiesis and favor the competing malignant stem cells, which could contribute to the poor donor engraftment and high incidence of disease relapse following allogeneic stem cell transplantation (SCT), the only curative treatment for patients with MPNs.2,6,8

Endothelial cells (ECs) are an essential component of the hematopoietic niche and most HSPCs reside close to a marrow sinusoid (the “perivascular niche”).9 MPNs are characterized by increased marrow angiogenesis compared to normal marrow.5-12 Although the existence and cell of origin of endothelial progenitors is still a subject of debate, JAK2V617F mutation can be detected in endothelial progenitors derived from the hematopoietic lineage (the so-called endothelial cell
colony-forming units; CFU-ECs or Hill) and, in some reports, in the true endothelial colony-forming cells (ECFC) based on in vitro assays.\textsuperscript{10,11} JAK2V617F mutation is also present in isolated liver or spleen ECs from patients with MPNs.\textsuperscript{12,13} Previously, we and others have shown that JAK2V617F-bearing ECs are critical in the development of the bleeding abnormalities in a murine model of JAK2V617F-positive MPNs in which JAK2V617F is expressed in all hematopoietic cells and endothelial cells.\textsuperscript{19} In addition, we have reported that the JAK2V617F-bearing vascular niche promotes the expansion of the JAK2V617F HSPCs in preference to JAK2V617FWT HSPCs.\textsuperscript{20,21} All of these observations suggest that ECs are involved in the pathogenesis of MPNs.

In the present study, using the hematopoietic and endothelial specific Tie2-Cre system and different marrow transplantation models, we demonstrate that JAK2V617F-mutant HSPCs are relatively protected by the JAK2V617F-bearing vascular niche from the otherwise lethal irradiation administered during conditioning for marrow transplantation. Taken together, our studies indicate that the mutant vascular niche could contribute to the poor donor cell engraftment and the high incidence of disease relapse well known to occur in patients with MPNs after allogeneic SCT. Therefore, targeting the altered hematopoietic vascular niche could provide more effective therapies for patients with MPNs.

Methods

Experimental mice

JAK2V617F Flip-Flop (FF1) mice\textsuperscript{22} were provided by Radek Skoda (University Hospital, Basel, Switzerland) and Tie2-Cre mice\textsuperscript{23} by Mark Ginsberg (University of California, San Diego, USA). The FF1 mice were crossed with Tie2-Cre mice to express JAK2V617F specifically in hematopoietic cells and ECs (Tie2/FF1 mice). All mice used were crossed onto a C57BL/6 background and were bred in a pathogen-free mouse facility at Stony Brook University. CD45.1\textsuperscript{+} congenic mice (SJL) were purchased from Taconic Inc. (Albany, NY, USA). Animal experiments were performed in accordance with the guidelines provided by the Institutional Animal Care and Use Committee.

Stem cell transplantation assays

The effects of the JAK2V617F-bearing vascular niche on MPN hematopoiesis were studied in vivo using marrow transplantation assays. First, we transplanted wild-type (WT) CD45.1 marrow cells into lethally irradiated (950 cGy)\textsuperscript{17} 8-14-week-old old Tie2/FF1 mice or WT controls (CD45.2). Peripheral blood was obtained every four weeks after transplantation, and CD45.1 donor chimerism and complete blood counts were measured.

To study the effects of HSPC JAK2V617F mutation on HSPC radioprotection, we generated a chimeric murine model with WT HSPCs and JAK2V617F-bearing vascular niche by transplanting WT marrow cells (CD45.1) into lethally irradiated (950cGy) Tie2/FF1 recipients (CD45.2). The transplantation of CD45.1 WT marrow cells into CD45.2 WT recipients served as a control. Following hematopoietic recovery and full donor cell engraftment, each set of mice were irradiated with 300cGy to create a radiation injury. Two hours later, marrow Lineageneg (Lin-) HSPCs were isolated using Lineage Cell Depletion Kit (Miltenyi Biotec, San Diego, CA, USA) for evaluation of cellular apoptosis and cell cycle status. For competitive marrow transplantation experiments, 5x10\textsuperscript{4} post-irradiated marrow cells (CD45.2) were injected intra-

venously together with 1x10\textsuperscript{5} competitor CD45.1 WT marrow cells into lethally irradiated (950 cGy) CD45.1 recipients. Peripheral blood was obtained every four weeks after transplantation, and CD45.2 chimerism was measured.

To study the effects of EC JAK2V617F mutation on HSPC radioprotection, we generated a chimeric murine model with WT HSPCs and JAK2V617F-bearing vascular niche by transplanting WT marrow cells (CD45.1) into lethally irradiated (950cGy) Tie2/FF1 recipients (CD45.2). The transplantation of CD45.1 WT marrow cells into CD45.2 WT recipients served as a control. Following hematopoietic recovery and full donor cell engraftment, each set of mice were irradiated with 300cGy to create a radiation injury. Two hours later, marrow Lineageneg (Lin-) HSPCs were isolated for evaluation of cellular apoptosis.

Additional details of the methods used can be found in the Online Supplementary Methods.

Results

Expression of JAK2V617F in Tie2\textsuperscript{+} cells protects marrow HSPCs from lethal irradiation

Mice expressing Cre under the control of the Tie2 promoter (Tie2-Cre) were crossed with JAK2V617F Flip-Flop (FF1) mice to generate mice bearing human JAK2V617F expression specifically in endothelial and hematopoietic cells (Tie2/FF1). The Tie2/FF1 mice develop an MPN-like phenotype with neutrophilia, thrombocytosis, significant splenomegaly, and greatly increased marrow vascular density, megakaryopoesis, and numbers of HSPCs.\textsuperscript{19,20}

To investigate the effects of the JAK2V617F-bearing vascular niche on MPN hematopoiesis in vivo, WT CD45.1 marrow cells were transplanted directly into lethally irradiated (950cGy) Tie2/FF1 mice or age-matched littermate control mice (CD45.2) (n=12 in each group) (Figure 1A). During a 3-month follow up, while all WT control recipients displayed full donor engraftment, 7 of 12 (approx. 60\%) Tie2/FF1 recipient mice displayed recovery of JAK2V617F-mutant hematopoiesis (mixed donor/recipient chimerism) ten weeks after transplantation (Figure 1B).

We followed some of the Tie2/FF1 and WT control recipients (n=7 in each group) for more than eight months. In contrast to the Tie2/FF1 recipients with full donor engraftment, the mixed chimeric mice developed neutrophilia, thrombocytosis, and splenomegaly (Figure 1C and D), similar to what has been observed in the primary Tie2/FF1 mice.\textsuperscript{19,20} Flow cytometry analysis revealed that JAK2V617F-mutant CD45.2 EPCR-CD48-CD150+ (E-SLAM) cells, which is a highly purified long-term repopulating HSPC population in normal and in MPN marrow,\textsuperscript{7,28} are significantly expanded in the mixed chimeric mice compared to Tie2/FF1 recipients with full donor engraftment or WT recipients (Figure 1E).

In virtually all our transplantation experiments performed over the past four years, we have used 950cGy radiation\textsuperscript{24} and have seen virtually 100\% donor engraftment in every recipient. In contrast, 7 of 12 mice in our Tie2/FF1 recipients of normal marrow demonstrated mixed chimerism with an average of 23\% recipient cells in peripheral blood at fourteen weeks following 950cGy irradiation and marrow transplantation, and developed an MPN phenotype resembling the primary Tie2/FF1 mice during more than eight months of follow up. These findings suggest that the JAK2V617F-mutant HSPCs in Tie2/FF1 mice are relatively protected from the otherwise...
Figure 1. JAK2V617F hematopoietic stem/progenitor cells (HSPCs) in the Tie2/FF1 mice are protected from lethal irradiation. (A) Scheme of direct marrow transplantation. (B) Peripheral blood CD45.1 chimerism following transplantation of wild-type (WT) CD45.1 marrow cells into lethally irradiated Tie2/FF1 mice or WT control mice (CD45.2) (n=12 in each group). (C) During more than eight months of follow up, Tie2/FF1 recipients with mixed chimerism developed both neutrophilia and thrombocytosis. (D) Spleens collected eight months following transplantation did not display significant weight differences between Tie2/FF1 recipients with full donor chimerism and WT recipients. In contrast, Tie2/FF1 recipients with mixed chimerism developed moderate splenomegaly compared to WT recipients (spleen weight 196 mg vs. 80 mg; P=0.010). (E) The frequency of normal marrow donor-derived E-SLAM (CD45.1) cells in the Tie2/FF1 recipients was unchanged from WT recipient mice. In contrast, JAK2V617F-mutant recipient-derived E-SLAM (CD45.2) cells were significantly expanded in Tie2/FF1 recipients with mixed chimerism. (F) Schematic diagram of irradiation and analysis of Tie2/FF1 and WT control mice. (G) Lin- HSPC cell apoptosis in Tie2-cre control or Tie2/FF1 mice after 300cGy irradiation (n=2 in each group). *P<0.05.
lethal irradiation administered during conditioning for marrow transplantation. To confirm this hypothesis, we irradiated primary Tie2/Ff1 mice or Tie2-Cre control mice with 300cGy, and two hours later their marrow Lin-HSPCs were isolated for evaluation of cellular apoptosis (Figure 1F). We found that the JAK2V617F HSPCs in the mutant vascular niche (i.e. Tie2/Ff1 mice) had significantly less cellular apoptosis compared to JAK2V617F HSPCs in WT vascular niche (i.e. control mice) (12.1% vs. 25.8%; P=0.043) (Figure 1G). Therefore, the JAK2V617F-mutant HSPCs in Tie2/Ff1 mice are relatively protected from lethal irradiation, which could be responsible for the reported high incidence of disease relapse in patients undergoing allogeneic SCT for MPNs.15,27

The JAK2V617F-mutant HSPC is more sensitive to radiation-induced apoptosis than JAK2WT HSPC

Tie2-Cre mice express Cre recombinase in both ECs and hematopoietic cells. To investigate whether the radioprotection phenotype noted in the prior experiments is due to the JAK2V617F mutation in Tie2/Ff1 HSPCs, we generated a chimeric murine model with JAK2V617F-mutant HSPCs and a wild-type (WT) vascular niche. Lin- marrow cells (CD45.2) were injected together with 1x10^5 competitor CD45.1 WT marrow cells into lethally irradiated (950 cGy) CD45.1 recipients (Figure 2A). Since the presence of JAK2V617F mutation in HSPCs may affect the cell's long-term proliferation, we focused on donor cell chimerism in the early phase of engraftment. During an 8-week post-transplant follow up, there was no difference in CD45.2 donor chimerism between the recipients of post-irradiated JAK2WT or JAK2V617F marrow cells (CD45.2) were injected together with 1x10^5 competitor CD45.1 WT marrow cells into lethally irradiated CD45.1 WT recipients (n=4 in each group). *P<0.05.

JAK2V617F-bearing ECs protect HSPCs from lethal irradiation

We next studied the effects of an EC JAK2V617F mutation on hematopoietic radioprotection. Lin- marrow HSPCs were isolated from WT or Tie2/Ff1 mice and cul-

---

Figure 2. The JAK2V617F-mutant hematopoietic stem/progenitor cell (HSPC) is more sensitive to radiation-induced apoptosis than JAK2WT HSPC. (A) Outline of experiment design to generate a chimeric murine model with JAK2V617F-mutant HSPCs and wild-type (WT) vascular niche. (B) After 300cGy irradiation, cell apoptosis (Annexin V+7AAD–) was significantly increased in JAK2V617F Lin- HSPCs (black bar) compared to JAK2WT Lin- HSPCs (gray bar). (C) Unirradiated JAK2V617F (black) Lin- HSPCs proliferated to a greater extent than JAK2WT (gray) Lin- HSPCs in vitro. (D) Peripheral blood donor chimerism following a competitive repopulation assay in which 5x10^5 post-irradiated JAK2WT or JAK2V617F marrow cells (CD45.2) were injected together with 1x10^5 competitor CD45.1 WT marrow cells into lethally irradiated CD45.1 WT recipients (n=4 in each group). *P<0.05.
tured on primary EC feeder layers derived from WT or Tie2/FF1 (JAK2V617F) murine lungs. The Lin- HSPC-EC co-cultures were irradiated with 300cGy ex vivo and cell number was counted within 24 hours of irradiation. We observed higher total cell numbers (1.6-fold; \( P = 0.026 \)) and hematopoietic progenitors (1.3-fold; \( P = 0.010 \)) from JAK2V617F HSPCs cultured on JAK2V617F-bearing ECs compared to their being cultured on JAK2WT ECs, suggesting that the JAK2V617F-bearing vascular niche contributes directly to JAK2V617F-mutant HSPC radioprotection (Figure 3A and B).

No significant difference was observed in cell numbers or hematopoietic progenitors between JAK2WT HSPCs cultured on JAK2V617F-bearing ECs and their being cultured on JAK2WT ECs in vitro after irradiation.

To further investigate the effects of JAK2V617F-bearing vascular niche on the response of HSPCs to radiation injury in vivo, we generated a chimeric murine model with WT HSPCs and mutant vascular niche by transplanting WT marrow cells (CD45.1) into lethally irradiated (950cGy) Tie2/FF1 recipients (CD45.2). The transplantation of CD45.1 WT marrow cells into CD45.2 WT recipients served as a control (Figure 3C). Based on our observation that Tie2/FF1 recipients did not develop any significant recovery of the JAK2V617F-mutant hematopoiesis until ten weeks after transplantation (Figure 1B), each set of chimeric mice were irradiated with 300cGy at 6-10 weeks following transplantation to create a radiation injury. Two hours later, marrow Lin- HSPCs were isolated for evaluation of cellular apoptosis. We found that the WT Lin- HSPC cell apoptosis was decreased in the JAK2V617F-mutant vascular niche compared to WT vascular niche (12.7\% vs. 19.7\%; \( P = 0.034 \)) (Figure 3D). Taken together, these data suggest that the JAK2V617F-bearing vascular niche contributes directly to HSPC radioprotection.

In contrast to our observations in vivo, where JAK2V617F-mutant HSPCs had increased apoptosis compared to JAK2WT HSPCs in the WT vascular niche (Figure 2A and B), cell numbers or progenitor numbers from the JAK2V617F HSPCs and JAK2WT HSPCs were similar when cultured on JAK2WT ECs (Figure 3A and B).

Similarly, while the JAK2V617F-bearing vascular niche is protective for WT HSPCs in vivo (Figure 3C and D), JAK2V617F EC did not significantly protect WT HSPC in vitro (Figure 3A and B). These results are likely due to the different cell-cell interactions and niche factors between the in vitro culture condition and in vivo microenvironment.

The JAK2V617F mutation alters vascular niche function to contribute to HSPC radioprotection

Next, we investigated how the JAK2V617F mutation alters EC function in the vascular niche to protect HSPCs from radiation injury. In our previous studies, we found that JAK2V617F-bearing ECs proliferate to a greater extent than JAK2WT ECs and display significantly increased
angiogenesis in vitro compared to JAK2WT ECs. In addition, the tubular structures formed by the JAK2V617F-bearing ECs in vitro were more stable than those from JAK2WT ECs. In this study, we found that JAK2V617F lung ECs displayed less cell apoptosis in vitro after 300cGy irradiation compared to JAK2WT ECs (7.7% vs. 19.5%; P=0.026) (Figure 4A). It has long been known that hematopoietic recovery following lethal irradiation requires an intact vasculature. Therefore, the increased cell proliferation and/or decreased apoptosis could expand the vascular niche in JAK2V617F-bearing mice, which in turn contributes to the hematopoietic radioprotection we have observed in the Tie2/FF1 recipient mice.

CXCL12 is an essential niche factor important for both HSPC maintenance and HSPC survival after radiation injury. Epidermal growth factor (EGF) and pleiotrophin (PTN), two other factors secreted by the vascular niche, have been shown to play important roles in the regulation of HSPC regeneration following radiation injury. Recently, we demonstrated that the expression level of CXCL12 was up-regulated in JAK2V617F-bearing marrow ECs compared to wild-type ECs, which could mediate the clonal expansion of JAK2V617F HSPCs, via the up-regulated CXCL12 receptor CXCR4, over JAK2WT HSPCs. To understand the EC signals responsible for HSPC radioprotection in the Tie2/FF1 recipient mice, we measured the expression levels of CXCL12, EGF, and PTN in both non-irradiated and irradiated JAK2WT and JAK2V617F lung ECs. qPCR analysis confirmed that there was upregulation of CXCL12 (2.5-fold; P=0.0001), EGF (4.0-fold; P=0.011) and PTN (11.4-fold; P=0.00001) in irradiated JAK2V617F-bearing ECs compared to irradiated JAK2WT ECs (Figure 4B and C). Furthermore, quantitative flow cytometry analysis showed that the proportion of marrow
CD150+/CD48+ HSPCs expressing phosphorylated EGFR was increased in irradiated JAK2V617F HSPCs (from Tie2/FF1 mice) as compared to irradiated JAK2WT HSPCs (from control mice) (2.7-fold; \( P=0.042 \)), suggesting that EGFR signaling activity was up-regulated in irradiated JAK2V617F HSPCs (Figure 4D). These results suggest that the JAK2V617F-bearing vascular niche contributes directly to HSPC radioprotection, possibly by its elaboration of soluble niche factors.

**Discussion**

Vascular ECs are a major component of the HSPC niche (the “vascular niche”) and provide many key factors that are required for HSPC maintenance. Patients with MPNs are characterized by increased marrow angiogenesis compared to normal marrow. ECs carrying the JAK2V617F mutation can be detected in patients with MPNs, suggesting that ECs are involved in the pathogenesis of MPNs. Here, by using the hematopoietic and endothelial specific Tie2-Cre system and different marrow transplantation models, we have been able to highlight the importance of JAK2V617F-bearing ECs in MPN disease relapse, which is seen in up to 40% of patients (especially after reduced intensity conditioning) following allogeneic SCT, the only curative treatment for MPNs.

It has long been known that hematopoietic recovery following lethal irradiation requires an intact vasculature. Following radiation injury, co-culture of irradiated HSPCs with ECs can rescue HSPCs with multilineage reconstitution capacity. Our previous study has demonstrated that JAK2V617F-bearing ECs proliferate to a greater extent than JAK2WT ECs in vitro. In this study, we show that the JAK2V617F-bearing ECs display less cell apoptosis in vitro after irradiation compared to JAK2WT ECs. In addition, the JAK2V617F-mutant Lin– HSPCs produce more cells and hematopoietic colonies after irradiation when cultured on JAK2V617F-bearing ECs compared to their being cultured on JAK2WT ECs. Moreover, the expression levels of CXCL12, EGF, and PTN, which are important niche factors involved in HSPC maintenance and/or HSPC regeneration following radiation injury, were up-regulated in irradiated JAK2V617F-bearing ECs compared to JAK2WT ECs (Figures 3 and 4). These results suggest that the JAK2V617F-bearing vascular niche contribute directly to HSPC radioprotection. Consistent with these findings, 7 of 12 mice in our Tie2/FF1 recipients of normal marrow demonstrated mixed chimerism of an average 77% donor in peripheral blood cells at fourteen weeks following transplantation (Figure 1B). Previously we reported that the JAK2V617F-bearing vascular niche promotes the expansion of the JAK2V617F HSPCs in preference to JAK2WT HSPCs and the development of marrow fibrosis. Since graft failure or poor graft function in MPN patients after SCT is most likely due to marrow fibrosis, our work has demonstrated that the mutant vascular niche can contribute to the poor donor cell engraftment and the high incidence of disease relapse, the two major causes of treatment-related morbidity and mortality associated with allogeneic SCT in patients with MPNs.

The Tie2-Cre mice express Cre recombinase in both ECs and hematopoietic cells. Although an EC-specific Cre (e.g. VEcadherin-Cre) would allow us to distinguish the specific role of ECs in HSPC radioprotection, we chose to use Tie2-Cre as it mimics the human MPNs in which both the HSPCs and ECs harbor the JAK2V617F mutation. In order to determine whether the radioprotection phenotype we have observed in the Tie2/FF1 mice is also due to the JAK2V617F mutation in HSPCs, we generated a chimeric murine model with JAK2V617F-mutant HSPCs and a WT vascular niche using marrow transplantation. We found that, in the WT vascular niche, the JAK2V617F-mutant Lin– HSPC is more (not less) sensitive to radiation-induced apoptosis than JAK2WT HSPCs. Although there have been reports that EC infusion could augment hematopoietic recovery following myeloablative injury, transplanted ECs exert their pro-regenerative effect transiently, and there is no evidence that donor marrow ECs could engraft and achieve long-term reconstitution in the recipient marrow vascular niche. Therefore, analysis six weeks post transplantation (Figure 2A and B) is unlikely to be affected by “carry-over” ECs from the Tie2/FF1 donor at the time of transplantation. In addition, the engraftment potential of irradiated JAK2V617F-mutant HSPCs does not differ from irradiated JAK2WT HSPCs (Figure 2). The results of these studies suggest that the radioprotection phenotype we have observed in the Tie2/FF1 recipients is unlikely to be due solely to the presence of the JAK2V617F mutation in HSPCs. In contrast, in another chimeric murine model with WT HSPCs and JAK2V617F-bearing vascular niche, WT Lin– HSPC is less sensitive to radiation-induced apoptosis in the JAK2V617F-mutant vascular niche compared to WT vascular niche (Figure 3). These results suggest that JAK2V617F-bearing vascular niche contributes directly to HSPC radioprotection.

We could not exclude the possibility that altered interactions between the JAK2V617F HSPCs and JAK2V617F ECs contribute to HSPC radioprotection in the Tie2/FF1 mice. Indeed, no significant difference was observed in cell numbers or hematopoietic progenitors between JAK2WT HSPCs cultured on JAK2V617F-bearing ECs compared to their being cultured on JAK2WT ECs after irradiation (Figure 3A and B). This observation suggests that the JAK2V617F-bearing vascular niche by itself may not be sufficient to account for the radioprotection phenotype. Rather, it is most likely that specific cell-cell interactions involving the stem cells and niche ECs are required to provide the radioprotection of JAK2V617F HSPCs when present in a JAK2V617F vascular niche, as exemplified by the up-regulated EGF-EGFR signaling reported in this study (Figure 4). Systemic analysis of HSPC and EC proteins using either quantitative proteomics or antibody-based arrays, along with specific knock-out mouse models would be required to further investigate the interactions between HSPCs and ECs in JAK2V617F-bearing MPNs in vitro and in vivo.

Although the JAK2V617F mutation has only been reported in liver and spleen ECs from patients with MPNs, it is very probably also present in their marrow ECs, considering that liver, spleen, and marrow are all hematopoietic organs during embryonic development and/or throughout adulthood. In most MPN patients, the stem cell compartment in MPN is heterogeneous with the presence of both JAK2 wild-type clones and the JAK2V617F mutant clones. We hypothesize that the vascular niche in MPN patients is also heterogeneous with the co-existence of both normal and mutant ECs. Since the JAK2V617F mutation is present in all HSPCs and ECs...
from birth in the Tie2/FF1 mice, our murine model may not present the same acquired clonality and heterogeneity vascular niche features that characterize patients with MPNs. Nonetheless, our study has demonstrated that the JAK2V617F-bearing vascular niche can protect the JAK2V617F HSPCs from the otherwise lethal irradiation administered during conditioning for marrow transplantation, which provides a mechanism for the high incidence of disease relapse in MPN patients after allogeneic SCT. The optimal conditioning regimen for MPN patients undergoing SCT has still not been determined. As most current conditioning regimens for SCT are not restricted to only radiation, further investigation using murine models with different quantities of mutant ECs versus WT ECs will be required to fully understand the effects of the JAK2V617F-bearing vascular niche on mutant HSPC expansion and HSPC resistance to lethal irradiation and the cytotoxic chemotherapies commonly used in SCT for patients with MPNs.

Acknowledgments

The authors thank Todd Ruel and Rebecca Connor (Flow Cytometry Core Facility, Stony Brook University, NY, USA) for their assistance with the flow cytometry experiments. We would also like to thank Dr. Yayo Ma (Stony Brook University, NY, USA) for his continuous support throughout this work. YZ is supported by the State Scholarship Fund from Chinese Scholarship Council.

Funding

This research was supported by the Veterans Affairs award IK2BX001559 (HZ) and National Heart, Lung, and Blood Institute grant R01 HL134970 (HZ).

References