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Deletion of Ptpn1 induces myeloproliferative neoplasm

Fatoumata Jobe¹, Bhumika Patel², Teodora Kuzmanovic², Hideki Makishima², Yue Yang¹, Bartłomiej Przychodzen², Robert E. Hutchison³, Kendra K. Bence⁴, Jaroslaw P. Maciejewski², and Golam Mohi¹

¹Department of Pharmacology, SUNY Upstate Medical University, Syracuse, NY, USA

²Department of Translational Hematology and Oncology Research, Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH, USA

³Department of Pathology, SUNY Upstate Medical University, Syracuse, NY, USA

⁴Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA, USA

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Deletion of chromosome 20q [del(20q)] is a common chromosomal abnormality associated with myeloid neoplasms including myeloproliferative neoplasms (MPN), myelodysplastic syndrome (MDS), MDS/MPN overlap disorders and acute myeloid leukemia (AML).^{1, 2} The del(20q) lesion is present in patients with MF at a high frequency (23%) and thus considered to be one of the most frequent cytogenetic abnormalities in MF.³ However, the identity of the target tumor-suppressor gene(s) within 20q involved in the pathogenesis of MF and other myeloid neoplasms remains elusive.

The *PTPN1* gene encoding protein tyrosine phosphatase non-receptor type 1 (PTPN1; also known as PTP1B) is located on human chromosome 20q13.1-q13.2. Both oncogenic and tumor suppressor functions for PTPN1 have been suggested. *PTPN1* is overexpressed in breast cancer and deletion of Ptpn1 inhibits ErbB2-induced breast tumorigenesis in mice.⁴ Somatic loss-of-function mutations in *PTPN1* have been associated with mediastinal B cell lymphoma and Hodgkin lymphoma.⁵ Moreover, ablation of Ptpn1 accelerates B-cell lymphomagenesis and decreases survival in p53-null mice.⁶ PTPN1 can negatively regulate JAK/STAT signaling,⁷ which is frequently found to be activated in MPNs.⁸ The common

Correspondence: Dr. Golam Mohi, Department of Pharmacology, SUNY Upstate Medical University, 750 East Adams Street, Syracuse, NY 13210, USA. Phone: 315-464-9861; Fax: 315-464-8014; mohim@upstate.edu.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

F Jobe performed research, analyzed data and wrote the manuscript; B Patel, T Kuzmanovic, H Makishima and B Przychodze collected and analyzed data; Y Yang analyzed data; RE Hutchison conducted histopathologic analysis and revised the manuscript; KK Bence provided study material; JP Maciejewski provided clinical specimens, analyzed data and revised the manuscript; G Mohi designed the research, analyzed data, and wrote the manuscript.

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occurrence of del(20q) involving the *PTPN1* locus in MPN and the negative regulatory role of the corresponding phosphatase in JAK/STAT signaling led us to hypothesize that *PTPN1* is a potential tumor-suppressor gene within 20q involved in MPN.

Using cytogenetic and single-nucleotide polymorphism (SNP) array-based karyotyping, we detected the presence of 20q deletion and assessed the status of the *PTPN1* gene locus in a cohort of 916 patients with myeloid malignancies (Supplementary Tables 1 and 2). The del(20q) involving *PTPN1* deletion was identified in 11/135 cases (8%) of sAML, 1/120 cases (1%) of pAML, 25/147 cases (17%) of MPN, 6/135 cases (4%) of MDS/MPN overlap disorders and 47/379 cases (12%) of MDS (Supplementary Figure 1). Thus, del(20q) involving *PTPN1* deletion was more commonly found in MPN in comparison to other myeloid neoplasms. We also determined that 22% of MF patients (25/116 cases) in our cohort had del(20q) with *PTPN1* deletion and 53% of MF patients (62/116 cases) were positive for the *JAK2V617F* mutation, 10% (12/116 cases) were positive for CALR mutation and 7% (8/116 cases) were positive for MPL mutation (Figure 1a). Among the 25 MF patients who had del(20q) with *PTPN1* deletion, 17 were also positive for the *JAK2V617F* mutation, 2 patients were positive for CALR mutation, while 6 other del(20q) patients did not have any of the known MPN-associated mutations (Supplementary Figure 2). Interestingly, 6 cases with various myeloid neoplasms had somatic microdeletion of the *PTPN1* locus (data not shown), but none of these cases had MF and coexisting MPN driver mutations. Fluorescence *in situ* hybridization (FISH) using a *PTPN1*-specific probe confirmed the presence of monoallelic deletion of *PTPN1* in MPN/MF bone marrow (BM) cells (Supplementary Figure 3). Overall, these data suggest that *PTPN1* deletion occurs in myeloid malignancies and is more frequently present in MPN/MF.

To test the effects of Ptpn1 deletion on hematopoiesis, we crossed Ptpn1 floxed (Ptpn1^{fl/fl}) mice⁹ with Mx1-Cre mice.¹⁰ At 4 weeks after birth, control (Ptpn1^{fl/fl}; no Cre) and Mx1-Cre;Ptpn1^{fl/fl} (referred as Ptpn1^{CKO}) mice were injected with 3 doses of 2 µg polyinosine-polycytosine (pI-pC) to induce Ptpn1 deletion in the mice hematopoietic compartments. As shown in Figure 1b, Ptpn1 was efficiently deleted in Ptpn1^{CKO} mice BM upon pI-pC induction. Compared to control animals, Ptpn1^{CKO} mice exhibited significantly increased white blood cells (WBC) and neutrophils in their peripheral blood (Figures 1c and d). However, no significant difference was observed in red blood cell (RBC) and platelet counts between control and Ptpn1^{CKO} mice (data not shown). Deletion of Ptpn1 also resulted in enlargement of spleen size/weight in Ptpn1^{CKO} mice (Figure 1e), suggesting that loss of Ptpn1 induces extramedullary hematopoiesis. Mice with heterozygous Ptpn1 deletion, however, did not exhibit any significant phenotype (data not shown).

Flow cytometric analyses showed significant expansion of myeloid (Gr-1⁺/Mac-1⁺) precursors in the BM and spleens of Ptpn1^{CKO} mice compared with control animals (Figure 1f and Supplementary Figure 4). Megakaryocytic (CD41⁺/CD61⁺) precursors were also significantly increased in the spleens of Ptpn1^{CKO} mice (Figure 1f and Supplementary Figure 4). Flow cytometric analyses also revealed significant increases in the numbers of LSK cells (Lin⁻Sca1⁺c-kit⁺) and its subsets including long-term hematopoietic stem cells (LT-HSC) and short-term HSC (ST-HSC) in Ptpn1^{CKO} mice compared with control animals (Figures 1g and Supplementary Figure 5a). Total numbers of common myeloid progenitors (CMP),

granulocyte-macrophage progenitors (GMP) and megakaryocyte-erythroid progenitors (MEP) were also significantly increased in Ptpn1^{ckO} mice (Figure 1h and Supplementary Figure 5b). Hematopoietic progenitor colony assays showed significant increases in CFU-GM colonies in the BM and spleens of Ptpn1^{ckO} mice compared with control animals (Figure 1i). We also observed significantly increased numbers of megakaryocytic (CFU-Mk) colonies in the BM of Ptpn1^{ckO} mice (Figure 1j). Together, these data suggest that loss of Ptpn1 promotes the expansion of HSC and increases myeloid/megakaryocytic differentiation.

Histopathologic analyses of the BM sections from Ptpn1^{ckO} mice showed increased granulocytic precursors with decreased erythroid precursors and mildly increased megakaryocytes (Figure 1k). Spleen sections from Ptpn1^{ckO} mice exhibited high myeloid to erythroid ratios and increased megakaryocytes. Reticulin staining demonstrated fibrosis (grade 2) in the BM and spleens of Ptpn1^{ckO} mice at 52 weeks after pI-pC induction, whereas control animals did not exhibit fibrosis at that age (Figure 1k). Together, these results suggest that deletion of Ptpn1 induces an MPN-like phenotype, which progresses to MF over time.

To determine whether the MPN/MF-like phenotype observed in Ptpn1^{ckO} mice was cell intrinsic, we transplanted BM cells from control and Ptpn1^{ckO} mice into lethally irradiated C57BL/6 wild-type recipient mice as outlined in Figure 2a. Compared with recipients of control BM (BMT-Control), transplanted animals receiving Ptpn1^{ckO} mice BM (BMT-Ptpn1^{ckO}) exhibited significantly increased WBC and neutrophil counts in their peripheral blood (Figures 2b and c). Histologic analysis confirmed the development of fibrosis in the BM and spleens of BMT-Ptpn1^{ckO} mice at 18 weeks after transplantation (Figure 2d). These results suggest that the observed effect of Ptpn1 deletion in the development of MPN/MF was cell-intrinsic.

To evaluate the effects of Ptpn1 deletion in hematopoietic stem cell reconstitution, we performed competitive repopulation assays as outlined in Figure 2e. The frequency and total number of donor-derived (CD45.2⁺) LSK cells was significantly increased in the BM and spleens of recipients of Ptpn1^{ckO} BM compared with recipients of control BM (Supplementary Figure 6a and Figure 2f). We also observed significantly greater percentages and increased numbers of donor-derived Gr-1⁺ myeloid cells in the BM and spleens of recipients of Ptpn1^{ckO} BM compared with control BM (Supplementary Figure 6b and Figure 2g). Together, these results suggest that loss of Ptpn1 increases the repopulating capacity of HSC and promotes expansion of myeloid lineage cells.

To investigate the effects of Ptpn1 deletion on hematopoietic signaling, we performed immunoblot analysis on total BM cell extracts from control and PTPN1^{ckO} mice. We observed markedly increased phosphorylation of STAT5, AKT and ERK in PTPN1^{ckO} BM compared to control BM cells (Figure 2h). We also observed enhanced phosphorylation of STAT5, AKT and ERK upon PTPN1 knockdown in human peripheral blood mononuclear cells as well as in murine hematopoietic Ba/F3 cells (Supplementary Figures 7 and 8a). In addition, PTPN1 knockdown significantly increased proliferation in Ba/F3 cells (Supplementary Figure 8b). Constitutive phosphorylation of JAK2/STAT5, AKT and ERK

has been frequently observed in MPN/MF.⁸ Moreover, STAT5, AKT and/or ERK signaling pathways have been shown to play important roles in the pathogenesis of MPN/MF.^{11–13} Therefore, it is plausible that enhanced phosphorylation/activation of STAT5, AKT and ERK caused by Ptpn1 deletion is likely to contribute to the development of MPN/MF-like phenotype in Ptpn1^{ckO} mice.

Consistent with previous reports suggesting that only heterozygous deletions of 20q are associated with myeloid malignancies,^{1, 2, 14} we observed monoallelic deletion of *PTPN1* in MPN/MF. Monoallelic deletion does not always correspond to 50% expression of the gene, as has been shown for other 20q target genes- *L3MBTL1*, *SGK2* and *MYBL2*, where heterozygous deletion results in almost complete loss or ~80% reduction in expression of these genes in patients.^{14, 15} We identified monoallelic *PTPN1* deletion in MPN patient-derived UKE-1 cell line carrying del(20q) (Supplementary Figure 9a). We observed more than 90% reduction in PTPN1 protein expression in UKE-1 cell line compared with MPN patient-derived SET-2 cell line expressing wild-type PTPN1 (Supplementary Figure 9a and b). Mechanisms involving aberrant methylation and/or elevated expression of microRNAs have been suggested to cause further reduction in expression of tumor-suppressor genes.^{14, 15} Future studies will determine if any such mechanisms exist in regulation of PTPN1 expression in patients with MPN/MF.

In summary, our data suggest a tumor suppressor role for PTPN1 in MPN/MF. However, we cannot exclude the existence of additional target genes in 20q involved in the pathogenesis of MPN/MF. It remains possible that haploinsufficiency of other target genes within 20q or additional genetic lesions outside of 20q may cooperate with *PTPN1* deletion in the pathogenesis of MPN/MF. We observed co-existence of *PTPN1* deletion and *JAK2V617F* mutation in significant cases of MF. Our preliminary data show that heterozygous or homozygous deletion of PTPN1 increases the severity of MPN in Jak2V617F knock-in mice. Future studies will determine whether loss of *PTPN1* cooperates with the *JAK2V617F* mutation in the pathogenesis of MPN.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Loss of *Ptpn1* induces MPN-like disease

(a) The frequency of *del(20q)* involving *PTPN1* deletion, JAK2, CALR and MPL mutations in MF patients is shown. (b) Immunoblot analysis shows efficient deletion of *Ptpn1* in the BM of *Ptpn1*^{KO} mice at 28 weeks after pI-pC induction. ERK2 was used as a loading control. (c) White blood cell (WBC) and (d) neutrophil (NE) counts in the peripheral blood of control (n=12) and *Ptpn1*^{KO} mice (n=15) at 4, 8, 12, 28 and 56 weeks after pI-pC induction. (e) Spleen size/weight was significantly increased in *Ptpn1*^{KO} mice compared with control animals at 28 weeks after pI-pC induction (n=9). Data are shown as mean ± SEM (* indicates *p*<0.05; ** indicates *p*<0.005 by Student's *t*-test). (f) Flow cytometric analysis of myeloid (Gr-1⁺/Mac-1⁺) and megakaryocytic (CD61⁺/CD41⁺) precursors in the BM and spleens from control and *Ptpn1*^{KO} mice at 28 weeks after pI-pC induction. Absolute numbers of myeloid and megakaryocytic precursors from control (n=5) and *Ptpn1*^{KO} (n=6) mice are shown in bar graphs as mean ± SEM. (g) Flow cytometric analysis of LSK (Lin⁻Sca1⁺c-kit⁺), LT-HSC (Lin⁻Sca1⁺c-kit⁺CD34⁻CD135⁻) and ST-HSC (Lin⁻Sca1⁺c-kit⁺CD34⁺CD135⁻) in the BM and spleens from control (n=5) and *Ptpn1*^{KO} mice (n=6). Total numbers of LSK, LT-HSC and ST-HSC are shown in bar graphs as mean ± SEM. (h) Total numbers of CMP (Lin⁻Sca1⁻c-kit⁺CD34⁺FcγR2/III^{low}), GMP (Lin⁻Sca1⁻c-kit⁺CD34⁺FcγR2/III^{high}) and MEP (Lin⁻Sca1⁻c-kit⁺CD34⁺FcγR2/III⁻) in the BM and

spleens from control (n=5) and Ptpn1^{ckO} mice (n=6) are shown in bar graphs as mean $\pm$ SEM. (i) CFU-GM colonies. BM or spleen cells from control (n=5) and Ptpn1^{ckO} mice (n=6) mice were plated in methylcellulose medium (M3434) containing cytokines. CFU-GM colonies were scored after 7 days. (j) CFU-Mk colonies. BM cells (1×10^5) from control and Ptpn1^{ckO} mice (n=3 to 5) were plated into collagen-based MegaCult medium supplemented with interleukin (IL)-3, IL-6, IL-11, and thrombopoietin (TPO). Megakaryocytic (CFU-Mk) colonies were assessed after culturing for 8 days. Data are shown as mean $\pm$ SEM (* indicates $p < 0.05$; ** indicates $p < 0.005$ by Student's *t*-test). (k) Histological analysis. Hematoxylin & eosin (H&E) staining of the BM sections from Ptpn1^{ckO} mice showed increased myeloid to erythroid ratios and mild increase in megakaryocytes. Spleens of Ptpn1^{ckO} mice showed extramedullary hematopoiesis, with a high myeloid to erythroid ratios and increase in both granulocytic and megakaryocytic cells. Reticulin staining showed fibrosis (grade 2 of 3) in the BM and spleens of Ptpn1^{ckO} mice at 52 weeks after Ptpn1 deletion. Scale bars, 20 μ m.

Figure 2. Deletion of Ptpn1 increases hematopoietic stem cell reconstitution and enhances hematopoietic signaling

(a) Experimental design for cell-autonomous bone marrow transplantation (BMT) assay. BM cells (1×10^6) from control and $Ptpn1^{cKO}$ mice were harvested at 24 weeks after pI-pC injection and transplanted into lethally irradiated wild type C57BL/6 recipient mice. (b) White blood cell (WBC) and (c) neutrophil (NE) counts in the peripheral blood of recipients of control (n=4) and $Ptpn1^{cKO}$ (n=5) mice BM at 4, 8, 12 and 16 weeks after BMT. (d) Hematoxylin & eosin (H & E) staining of the BM sections from BMT- $Ptpn1^{cKO}$ mice showed fibrotic-appearing marrow with increased myeloid to erythroid ratio. Spleens of BMT- $Ptpn1^{cKO}$ mice showed disordered architecture and increased myeloid to erythroid ratio, whereas spleens of BMT-Control mice appeared normal. Reticulin staining showed fibrosis in the BM and spleens of BMT- $Ptpn1^{cKO}$ mice at 18 weeks after transplantation. BM and spleens of BMT-Control mice did not exhibit fibrosis. Scale bars, 20 μ m. (e) Experimental design for competitive reconstitution assay. BM cells (5×10^5) from control or $Ptpn1^{cKO}$ mice (CD45.2⁺) (at 14 weeks after pI-pC induction) were mixed with wild type CD45.1⁺ mice BM (5×10^5) at a 1:1 ratio and transplanted into lethally irradiated CD45.1⁺ recipient mice. The recipient mice were analyzed at 16 weeks after transplantation. Representative flow cytometry plots and the total numbers of donor-derived (CD45.2⁺) LSK

cells (**f**) and donor-derived Gr-1⁺ myeloid cells (**g**) in the BM and spleens of recipient animals at 16 weeks after transplantation are shown; bar graphs represent mean $\pm$ SEM. (Control, n=4; Ptpn1^{ckO}, n=4). Student's *t*-test was used to compare between two groups of mice (* indicates $p<0.05$; ** indicates $p<0.005$). (**h**) Total BM cell extracts from primary control and Ptpn1^{ckO} mice were immunoblotted with phospho-specific or total antibodies against STAT5, AKT and ERK. β -actin was used as a loading control. Representative blots from four independent experiments are shown.