MYELOID NEOPLASIA

The CSF3R T618I mutation causes a lethal neutrophilic neoplasia in mice that is responsive to therapeutic JAK inhibition

Angela G. Fleischman,1,2 Julia E. Maxson,1,2 Samuel B. Luty,1,2 Anupriya Agarwal,1 Lacey R. Royer,1,2 Melissa L. Abel,1,2 Jason D. MacManiman,1,2 Marc M. Loriaux,1,2 Brian J. Druker,1,2,3 and Jeffrey W. Tyner1,2,4
1Division of Hematology & Medical Oncology, 2Knight Cancer Institute, 3Howard Hughes Medical Institute, and 4Department of Cell & Developmental Biology, Oregon Health & Science University, Portland, OR

Key Points

- CSF3R T618I is sufficient to drive a lethal myeloproliferative disease in a mouse bone marrow transplant model.
- Treatment of CSF3R T618I mice with the JAK1/2 inhibitor ruxolitinib effectively reduces white blood cell count and spleen size.

Introduction

We have recently identified targetable mutations in CSF3R (GCSFR) in 60% of chronic neutrophilic leukemia (CNL) and atypical (BCR-ABL−negative) chronic myeloid leukemia (aCML) patients. Here we demonstrate that the most prevalent, activating mutation, CSF3R T618I, is sufficient to drive a lethal myeloproliferative disorder in a murine bone marrow transplantation model. Mice transplanted with CSF3R T618I–expressing hematopoietic cells developed a myeloproliferative disorder characterized by overproduction of granulocytes and granulocytic infiltration of the spleen and liver, which was uniformly fatal. Treatment with the JAK1/2 inhibitor ruxolitinib lowered the white blood count and reduced spleen weight. This demonstrates that activating mutations in CSF3R are sufficient to drive a myeloproliferative disorder resembling aCML and CNL that is sensitive to pharmacologic JAK inhibition. This murine model is an excellent tool for the further study of neutrophilic myeloproliferative neoplasms and implicates the clinical use of JAK inhibitors for this disease. (Blood. 2013;122(22):3628-3631)

Methods

Expression vectors

Human CSF3R transcript variant 1 (NM_000760.2) pDONR vector was purchased from GeneCopoeia. CSF3R T618I mutation was made using the QuickChange II XL site-directed mutagenesis kit (Agilent Technologies). The Gateway Cloning System (Invitrogen) was used to clone CSF3R WT and CSF3R T618I into the MSCV-IRES-green fluorescent protein (GFP) plasmid.

The online version of this article contains a data supplement. The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 USC section 1734.

© 2013 by The American Society of Hematology
Bone marrow transplantation

Wild-type BALB/C mice (000651) were purchased from Jackson Labs. Retroviral infection and transplantation was performed as previously described.8 All mouse work was performed with approval from the Oregon Health & Science University Institutional Animal Care and Use Committee.

Ruxolitinib treatment

Mice were administered 90 mg/kg ruxolitinib phosphate (ChemScene) dissolved in 5% dimethyl acetamide, 0.5% methylcellulose, or vehicle alone by oral gavage twice daily as previously described.9

Flow cytometry

After red blood cell lysis, cells were stained with the following antibodies for 20 minutes at 4°C: PE-CD3 clone 145-2C11 (eBioscience), PerCP Cy5.5-CD19 clone HIB1g (BD PharMingen), APC-CD11b clone M1/70 (eBioscience), and E450-Gr-1 clone RB6-8C5 (eBioscience). All flow cytometry was performed on an Aria III (BD Biosciences). Data were analyzed using FlowJo software (TreeStar).

Phospho-flow cytometry

Peripheral blood was collected from live animals immediately into fix/lyse buffer (BD Biosciences) for 15 minutes at 37°C. Cells were then permeabilized with methanol and stained with PE-pSTAT3 (pY705) (BD Biosciences) and analyzed by flow cytometry.

Pathology

Spleens, livers, and femurs were removed at necropsy and fixed in 10% zinc formalin. Fixed tissues were sectioned and stained using hematoxylin and eosin by the Histopathology Shared Resource at Oregon Health & Science University.

Results and discussion

CSF3R T618I causes a lethal myeloproliferative disorder resembling neutrophilic leukemia

To determine whether CSF3R T618I is sufficient to drive neoplastic expansion of neutrophils, we transplanted bone marrow expressing CSF3R T618I or CSF3R WT into irradiated mice. CSF3R WT was chosen for comparison to control for any effects of ectopic CSF3R expression. Blood counts were monitored 1 to 2 times per week (Figure 1A). The CSF3R T618I mice had an initial transient leukocytosis predominantly comprised of granulocytes (Figure 1A-C), trending back to normal by day 33 post transplant. The initial leukocytosis was specific to CSF3R T618I mice, indicating that it is a direct effect of the mutation rather than simply overexpression of CSF3R. At day 47, the CSF3R T618I mice had a dramatic rise in WBCs, again comprised of predominantly mature granulocytes (Figure 1A-C; supplemental Figure 1, available on the Blood Website). By day 90, this leukocytosis was persistent and uniformly fatal in mice transplanted.
with hematopoietic cells expressing CSF3R<sup>T618I</sup> (Figure 1D). Expression of CSF3R<sup>WT</sup> did not lead to significant leukocytosis or morbidity in any mice. CSF3R<sup>T618I</sup> activated signaling downstream of the receptor, leading to an increase in phospho-STAT3 relative to mice expressing CSF3R<sup>WT</sup> (Figure 1E).

**CSF3R<sup>T618I</sup> causes granulocytic expansion in the bone marrow and infiltration of granulocytes in the spleen and liver**

Bone marrow of both CSF3R<sup>T618I</sup> and CSF3R<sup>WT</sup> mice exhibited abnormal hypercellularity; this was most pronounced in the CSF3R<sup>T618I</sup> mice (supplemental Figure 2). The marrow was composed mostly of mature granulocytes (supplemental Figure 2). CSF3R<sup>T618I</sup> and CSF3R<sup>WT</sup> mice also had infiltration of mature granulocytes into the spleen and liver (supplemental Figure 2), which was more pronounced in the CSF3R<sup>T618I</sup> than the CSF3R<sup>WT</sup> mice (supplemental Figure 2). The ability of CSF3R<sup>T618I</sup> as well as CSF3R<sup>WT</sup> to enhance production of mature myeloid cells is consistent with the normal physiological role of CSF3R in promoting neutrophil production and maturation. The T618I mutation in CSF3R further augments this signaling—possibly by conferring ligand independence to the receptor<sup>3</sup>—resulting in a more exaggerated proliferation of granulocytes. The dramatic granulocytic expansion in mice transplanted with cells expressing CSF3R<sup>T618I</sup> led to death in all cases, whereas the granulocytic expansion in the CSF3R<sup>WT</sup> mice was not sufficient to cause death.

The JAK1/2 inhibitor ruxolitinib decreases WBC count and reduces splenomegaly in CSF3R<sup>T618I</sup> mice

We previously demonstrated that activating CSF3R mutations lead to preferential downstream signaling via JAK kinases, and a CNL patient carrying a JAK activating CSF3R<sup>T618I</sup> mutation showed marked clinical improvement after administration of the JAK1/2 inhibitor ruxolitinib.<sup>1</sup> To determine whether the granulocytic expansion seen in CSF3R<sup>T618I</sup> mice is dependent upon the JAK kinase pathway, we tested the effect of ruxolitinib in a second cohort of CSF3R<sup>T618I</sup> mice. Oral administration of ruxolitinib (90 mg/kg × 2/d) or vehicle was started at day 12 post transplant, at which time mice already exhibited leukocytosis. Ruxolitinib treatment resulted in a prompt reduction in WBC count and a decrease in spleen weight (Figure 2A-C). Consistent with its ability to improve constitutional symptoms such as fatigue and early satiety in myelofibrosis,<sup>12,13</sup> ruxolitinib-treated mice had increased body weight compared with vehicle-treated mice (Figure 2D). This demonstrates that the pathologic expansion of granulocytes in the CSF3R<sup>T618I</sup> mouse model is sensitive to JAK inhibition and warrants further investigation into the therapeutic use of JAK inhibitors in patients with CNL harboring the CSF3R<sup>T618I</sup> mutation.

Here we demonstrate that expression of CSF3R<sup>T618I</sup> in hematopoietic cells is sufficient to drive a lethal granulocytic expansion in mice. These data, in addition to the high prevalence of the CSF3R<sup>T618I</sup> mutation in patients with CNL,<sup>1,2</sup> implicate CSF3R<sup>T618I</sup> as an important genetic driver of neutrophilic leukemias. Moreover, disease in the mouse model responds to therapeutic JAK inhibition, consistent with our previously observed disease response in a CNL patient treated with ruxolitinib.<sup>1</sup> The CSF3R<sup>T618I</sup> bone marrow transplant provides a tractable mouse model of neutrophilic leukemia that will be useful for further studying the underlying biology of and therapeutic interventions in neutrophilic myeloproliferative neoplasms.

**Acknowledgments**

The authors thank the histopathology shared resource at the Oregon Health & Science University for preparing the histology slides and performing hematoxylin and eosin staining.

This work was supported in part by The Leukemia & Lymphoma Society, the Training Program in Molecular Hematology (ST32HL007781), a Leukemia & Lymphoma Society Fellow Award, and a Medical Research Foundation Early Clinical Investigator Award (J.M.); and grants from the V Foundation for Cancer
Research, the William Lawrence and Blanche Hughes Fund, the Gabrielle’s Angel Foundation for Cancer Research, and the National Cancer Institute, National Institutes of Health (5 R00CA151457-04) (J.W.T.).

Authorship


Conflict-of-interest disclosure: J.W.T. and A.G.F. both receive funding for administration of clinical trials from Incyte, manufacturer of ruxolitinib. The other authors declare no competing financial interests.

The current affiliation for A.G.F. is Division of Hematology/Oncology, University of California-Irvine, Irvine, CA.

Correspondence: Jeffrey W. Tyner, Oregon Health & Science University, BRB 511, Mailcode L592, 3181 SW Sam Jackson Park Rd, Portland, OR 97239; e-mail: tynerj@ohsu.edu.

References

The CSF3R T618I mutation causes a lethal neutrophilic neoplasia in mice that is responsive to therapeutic JAK inhibition

Angela G. Fleischman, Julia E. Maxson, Samuel B. Luty, Anupriya Agarwal, Lacey R. Royer, Melissa L. Abel, Jason D. MacManiman, Marc M. Loriaux, Brian J. Druker and Jeffrey W. Tyner