THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 290, NO. 31, pp. 19093–19103, July 31, 2015

Deficiency of β Common Receptor Moderately Attenuates the Progression of Myeloproliferative Neoplasm in *Nras*$^{G12D/+}$ Mice*

Received for publication, March 24, 2015, and in revised form, June 12, 2015 Published, JBC Papers in Press, June 16, 2015, DOI 10.1074/jbc.M115.653154

Jingfang Zhang[‡], **Erik A. Ranheim**[§], **Juan Du**[‡], **Yangang Liu**[‡], **Jinyong Wang**[‡], **Guangyao Kong**[‡], **and Jing Zhang**[‡1]

From the [‡]*McArdle Laboratory for Cancer Research, University of Wisconsin-Madison, Madison, Wisconsin 53705 and the* [§]*Department of Pathology and Laboratory Medicine, University of Wisconsin School of Medicine and Public Health, University of Wisconsin Carbone Cancer Center, Madison, Wisconsin 53705*

Background: GM-CSF signaling is important in establishing and maintaining juvenile/chronic myelomonocytic leukemia (JMML/CMML).
Results: The common β chain of GM-CSF receptor (βc) is dispensable for the function of CMML-initiating cells, but βc$^{-/-}$ prolongs the survival of CMML-bearing mice.
Conclusion: βc$^{-/-}$ slows down CMML progression but does not abrogate its initiation.
Significance: Inhibiting GM-CSF signaling might alleviate JMML/CMML symptoms but would not eradicate the disease.

Activating Ras signaling is a major driver in juvenile and the myeloproliferative variant of chronic myelomonocytic leukemia (JMML/MP-CMML). Numerous studies suggest that GM-CSF signaling plays a central role in establishing and maintaining JMML/MP-CMML phenotypes in human and mouse. However, it remains elusive how GM-CSF signaling impacts on JMML/MP-CMML initiation and progression. Here, we investigate this issue in a well characterized MP-CMML model induced by endogenous *Nras*$^{G12D/+}$ mutation. In this model, *Nras*$^{G12D/+}$ hematopoietic stem cells (HSCs) are required to initiate and maintain CMML phenotypes and serve as CMML-initiating cells. We show that the common β chain of the GM-CSF receptor (βc) is dispensable for *Nras*$^{G12D/+}$ HSC function; loss of βc does not affect the expansion, increased self-renewal, or myeloid differentiation bias in *Nras*$^{G12D/+}$ HSCs. Therefore, βc$^{-/-}$ does not abrogate CMML in *Nras*$^{G12D/+}$ mice. However, βc deficiency indeed significantly reduces *Nras*$^{G12D/+}$-induced splenomegaly and spontaneous colony formation and prolongs the survival of CMML-bearing mice, suggesting that GM-CSF signaling plays an important role in promoting CMML progression. Together, our results suggest that inhibiting GM-CSF signaling in JMML/MP-CMML patients might alleviate disease symptoms but would not eradicate the disease.

Juvenile and chronic myelomonocytic leukemia (JMML[2] and CMML) belong to the group of "mixed myelodysplastic/myeloproliferative diseases" (1, 2). CMML primarily occurs in the elderly with median ages at presentation ranging from 65 to 75 years, whereas JMML exclusively affects children, typically under the age of 4 years. Despite the demographic difference, CMML and JMML share similar clinical features, including monocytosis, hepatosplenomegaly, and the absence of the *BCR-ABL* fusion gene. At the molecular level, activating Ras signaling is a central theme in JMML and in the myeloproliferative variant of CMML (MP-CMML) (1, 3–6). Consistent with human studies, mice harboring an oncogenic *Ras* allele and mice deficient for *Nf1*, a negative regulator of Ras signaling, develop JMML/MP-CMML-like phenotypes (7–15).

A cellular characteristic of both JMML and CMML is the formation of excess numbers of CFU-GM colonies in semisolid cultures in the absence and presence of subsaturating concentrations of GM-CSF (16–18). GM-CSF is an important hematopoietic cytokine that regulates the survival, proliferation, differentiation, and activation of various hematopoietic cell types, especially macrophages and granulocytes (19). Upon binding to its heteromeric receptor, which is composed of α (GM-CSFRα) and β subunit, the biological activities of GM-CSF are exerted through Jak2/Stat5 and Ras/Raf/MEK/ERK pathways (20). Despite the hypersensitivity of JMML/CMML myeloid progenitors to GM-CSF, mice deficient for GM-CSF or its receptor develop normally and show no significant alterations of hematopoiesis (19, 20), suggesting that GM-CSF signaling is dispensable for normal hematopoiesis. Therefore, antagonizing GM-CSF signaling has been explored as a potential strategy for treating JMML/MP-CMML.

Several studies suggest that GM-CSF signaling plays a central role in establishing and maintaining JMML/MP-CMML-like phenotypes. In a recent study of GM-CSF signaling in JMML and CMML patient samples, an aberrant Stat5 signaling signa-

* This work was supported by National Institutes of Health Grants R01 CA152108 and HL113066 and a Scholar Award from the Leukemia & Lymphoma Society (to J. Z.). This work was also supported in part by National Institutes of Health NCI Grant P30 CA014520 through the University of Wisconsin Comprehensive Cancer Center. The authors declare that they have no conflicts of interest with the contents of this article.

[1] To whom correspondence should be addressed: Room 7453, McArdle Laboratory, 1111 Highland Ave., University of Wisconsin-Madison, Madison, WI 53705. Tel.: 608-263-1147; Fax: 608-262-2824; E-mail: zhang@oncology.wisc.edu.

[2] The abbreviations used are: JMML, juvenile myelomonocytic leukemia; CMML, chronic myelomonocytic leukemia; MP-CMML, myeloproliferative variant of CMML; MP, myeloid progenitor; HSC, hematopoietic stem cell; βc, common β chain of GM-CSF receptor; pI-pC, polyinosinic-polycytidylic acid; EdU, 5-ethynyl-2′-deoxyuridine; AML, acute myeloid leukemia; m, mouse; p, phospho.

ture was identified in a subpopulation of monocytic cells defined as $CD33^+$ $CD14^+$ $CD34^-$ $CD38^{lo}$ cells (21). This subset of cells can be used to monitor disease status at diagnosis, remission, relapse, and malignant transformation to an acute phase. Consistent with this notion, we found that recipients transplanted with $Nras^{G12D/+}$ cells acquire hypersensitivity to GM-CSF (both the Jak2/Stat5 and the Ras/Raf/MEK/ERK pathways are hyperactivated upon GM-CSF stimulation) during MP-CMML progression, which in some cases is attributed to uniparental disomy of the oncogenic *Nras* allele (10). Short-term inhibition of GM-CSF not only induces remission of JMML in engrafted immunodeficient mice (22) but also achieves transient clinical response in an end-stage JMML patient (23).

The long-term effects of inhibiting GM-CSF on JMML are evaluated in the $Nf1^{-/-}$ model. Four of six $Gmcsf^{-/-}$ mice transplanted with $Nf1^{-/-}$; $Gmcsf^{-/-}$ fetal liver cells develop JMML-like phenotypes with prolonged latency (24), which is postulated to be due to the residual activity of pre-formed GM-CSF receptor in the absence of GM-CSF (25). Subsequently, a study using the *Mx1-Cre* transgene to inactivate a conditional *Nf1* allele in $\beta c^{-/-}$ hematopoietic cells shows that the severity of JMML-like phenotypes is reduced but not abrogated, whereas mice transplanted with $Nf1^{-/-}$; $\beta c^{-/-}$ stem cells do not develop significant JMML over 1 year (26). Therefore, it remains elusive how GM-CSF signaling impacts on JMML/MP-CMML initiation and progression.

We addressed this question using the well characterized MP-CMML model induced by oncogenic *Nras* (10–12, 27, 28). In this model, acute expression of oncogenic *Nras* from its endogenous locus leads to expanded hematopoietic stem cell (HSC) compartment, increased HSC self-renewal, and myeloid differentiation bias in mutant HSCs. The myeloid progenitor (MP) compartment is expanded without significant hyperactivation of GM-CSF signaling or hyperproliferation in these cells, suggesting that the increased number of MPs is largely due to the increased myeloid differentiation potential of $Nras^{G12D/+}$ HSCs. Consistent with this notion, ~95% of recipients transplanted with $Nras^{G12D/+}$ bone marrow cells develop MP-CMML-like phenotypes after a long latency. $Nras^{G12D/+}$ HSCs are required to initiate and maintain the disease phenotypes and thus serve as CMML-initiating cells, whereas MPs acquire secondary genetic hits to gain hypersensitivity to GM-CSF and push monocytosis to develop *in vivo*.

We chose a genetic approach to stably delete GM-CSF signaling in $Nras^{G12D/+}$ mice. The GM-CSF receptor shares a common β subunit (βc) with IL-3 and IL-5 receptors (29). The GM-CSFRα and IL-5 receptor α subunits only pair with βc, whereas IL-3 receptor α subunit forms heterodimers with both βc and an IL-3-specific β subunit in mouse (30). Therefore, $\beta c^{-/-}$ bone marrow cells respond to IL-3 but not GM-CSF. Here, we report that $Nras^{G12D/+}$; $\beta c^{-/-}$ HSCs are very similar as $Nras^{G12D/+}$ HSCs; both of them show comparably increased numbers, increased self-renewal, and increased myeloid differentiation bias resulting in an expanded MP compartment. These results suggest that βc is dispensable for $Nras^{G12D/+}$ HSCs, the CMML-initiating cells. Therefore, deletion of βc does not abrogate CMML formation in recipients transplanted with $Nras^{G12D/+}$ cells. However, loss of βc-mediated GM-CSF signaling indeed attenuates CMML progression as demonstrated by reducing splenomegaly, abolishing spontaneous colony formation, and prolonging the survival of recipients with $Nras^{G12D/+}$ cells. Our data suggest that inhibiting GM-CSF signaling in JMML/MP-CMML patients might provide transient symptomatic improvements but would not eradicate the disease.

Materials and Methods

Mice—All mouse lines were maintained on a pure C57BL/6 genetic background (>N10). The conditional $Nras^{LSL\ G12D/+}$ allele is described in Ref. 11. $Nras^{LSL\ G12D/+}$ mice were crossed to Mx1-Cre mice to generate mice carrying both alleles ($Nras^{LSL\ G12D/+}$; *Mx1-Cre*). The common β subunit knock-out mice ($\beta c^{-/-}$) were obtained from The Jackson Laboratory (stock number 005940). $Nras^{LSL\ G12D/+}$; $\beta c^{+/-}$ mice were crossed to *Mx1-Cre*; $\beta c^{-/-}$ to generate compound mice $Nras^{LSL\ G12D/+}$; *Mx1-Cre*; $\beta c^{-/-}$. Genotyping of $Nras^{G12D/+}$ and *Mx1-Cre* was done as described previously (10). Genotyping of βc was performed per the instructions of The Jackson Laboratory. $CD45.1^+$ congenic recipient mice were purchased from the NCI, National Institutes of Health.

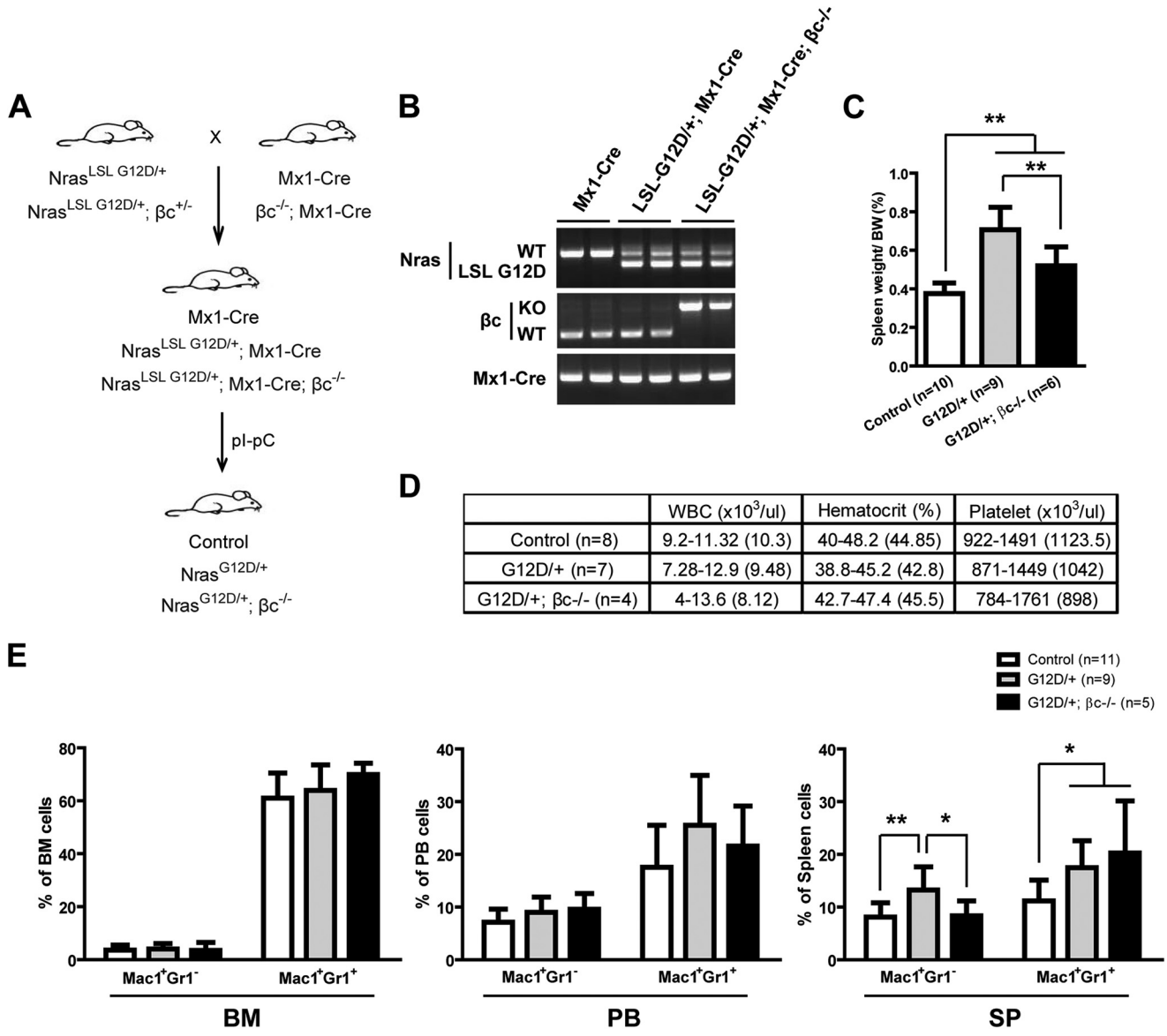
To induce Cre expression, 5–7-week-old mice were injected intraperitoneally with 100 μg of polyinosinic-polycytidylic acid (pI-pC; GE Healthcare) every other day for two doses. The day of first pI-pC injection was defined as Day 1. All animal experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health) and approved by an Animal Care and Use Committee at the University of Wisconsin-Madison. The program is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care.

Murine Bone Marrow Transplantation—2.5×10^5 total bone marrow cells ($CD45.2^+$) were mixed with the same number of congenic bone marrow cells ($CD45.1^+$) and injected into individual lethally irradiated mice as described previously (10).

Flow Cytometric Analysis of Hematopoietic Tissues—For lineage analysis of peripheral blood, bone marrow, and spleen, flow cytometric analyses were performed as described previously (9). HSCs, multipotent progenitors, Lin^- $Sca1^+$ $cKit^+$ (LSK), and MPs in bone marrow and spleen were analyzed as described previously (12, 27). Stained cells were analyzed on a FACSCalibur or LSRII (BD Biosciences).

Directly conjugated or biotin-conjugated antibodies specific for the following surface antigens were purchased from eBioscience: CD45.1 (A20), CD45.2 (104), Mac-1 (M1/70), Gr-1 (RB6-8C5), CD3 (145-2C11), CD4 (RM4-5), CD8 (53-6.7), CD19 (eBio1D3), Thy1.2 (53-2.1), TER119 (TER-119), B220 (RA3-6B2), IgM (eB121-15F9), IL-7Rα (B12-1), CD41 (eBioMWReg30), CD48 (HM48-1), Sca1 (D7), cKit (2B8), and CD34 (RAM34). FcγRII/III (2.4G2) was purchased from BD Biosciences. CD150 (TC15-12F12.2) was purchased from BioLegend.

Colony Assay—A total of 5×10^4 bone marrow cells were plated in duplicate in semi-solid medium MethoCult M3234 (StemCell Technologies) supplemented with mouse GM-CSF or IL-3 (PeproTech, Rocky Hill, NJ) according to the manufa-



	WBC (x10^3/ul)	Hematocrit (%)	Platelet (x10^3/ul)
Control (n=8)	9.2-11.32 (10.3)	40-48.2 (44.85)	922-1491 (1123.5)
G12D/+ (n=7)	7.28-12.9 (9.48)	38.8-45.2 (42.8)	871-1449 (1042)
G12D/+; βc-/- (n=4)	4-13.6 (8.12)	42.7-47.4 (45.5)	784-1761 (898)

FIGURE 1. **Loss of βc decreases *Nras$^{G12D/+}$*-induced splenomegaly.** Mice were treated with pI-pC and euthanized on Day 12 for analysis as described under "Materials and Methods." *A*, schematic illustration of the strategy to generate experimental mice. *B*, genotyping analysis of genomic DNA to detect different alleles in representative control, *Nras$^{LSLG12D/+}$*, and *Nras$^{LSLG12D/+}$; Mx1-Cre; βc$^{-/-}$* mice. *C*, the ratio of spleen weight to body weight (*BW*) of control, *Nras$^{G12D/+}$*, and *Nras$^{LSLG12D/+}$; βc$^{-/-}$* mice. *D*, complete blood count was performed on peripheral blood samples collected from control, *Nras$^{G12D/+}$*, and *Nras$^{G12D/+}$; βc$^{-/-}$* mice. The range and median of the data are shown. *E*, flow cytometry analysis of bone marrow (*BM*), peripheral blood (*PB*), and spleen (*SP*) cells using myeloid lineage-specific markers. Data are presented as mean ± S.D. *, $p < 0.05$; **, $p < 0.01$.

cturer's protocol. The colonies were counted after 7–10 days in culture.

Flow Cytometric Analysis of Phospho-ERK and Phospho-Stat5—Phosphorylated ERK1/2 and STAT5 were analyzed in defined Lin$^{-/low}$ cKit$^+$ and Lin$^{-/low}$ cKit$^-$ cells essentially as described previously (10). Surface proteins were detected with FITC-conjugated antibodies (eBioscience) against B220 (RA3–6B2), Gr-1 (RB6-8C5), CD3 (145–2C11), CD4 (GK1.5), CD8 (53-6.7), and TER119, and phycoerythrin-conjugated anti-CD117/cKit antibody (2B8). p-ERK1/2 was detected by a primary antibody against p-ERK (Thr-202/Tyr-204; Cell signaling Technology) followed by allophycocyanin-conjugated donkey anti-rabbit F(ab′)2 fragment (Jackson ImmunoResearch Laboratories). p-Stat5 (p-Tyr-694) was detected by Alexa Fluor 647-conjugated primary antibody against phospho-Stat5 (BD Biosciences).

EdU Incorporation—EdU (Invitrogen) was administered as a single dose of 1 mg injected intraperitoneally. EdU incorporation was measured 16 h later using the Click-It EdU Pacific Blue flow kit (Invitrogen) as described previously (31). Briefly, Sca1$^+$ cells were enriched using an AutoMACS (Miltenyi). Enriched cells were first stained with FITC-conjugated antibodies against CD41, CD48, B220, TER119, Gr1, and allophycocyanin-conjugated CD150. After Click-It reaction, cells were then stained with phycoerythrin-conjugated c-Kit and PerCP Cy5.5-Sca1. The stained cells were analyzed on an LSRII (BD Biosciences).

Complete Blood Count and Histopathology—Complete blood count analysis was performed using a Hemavet 950FS (Drew

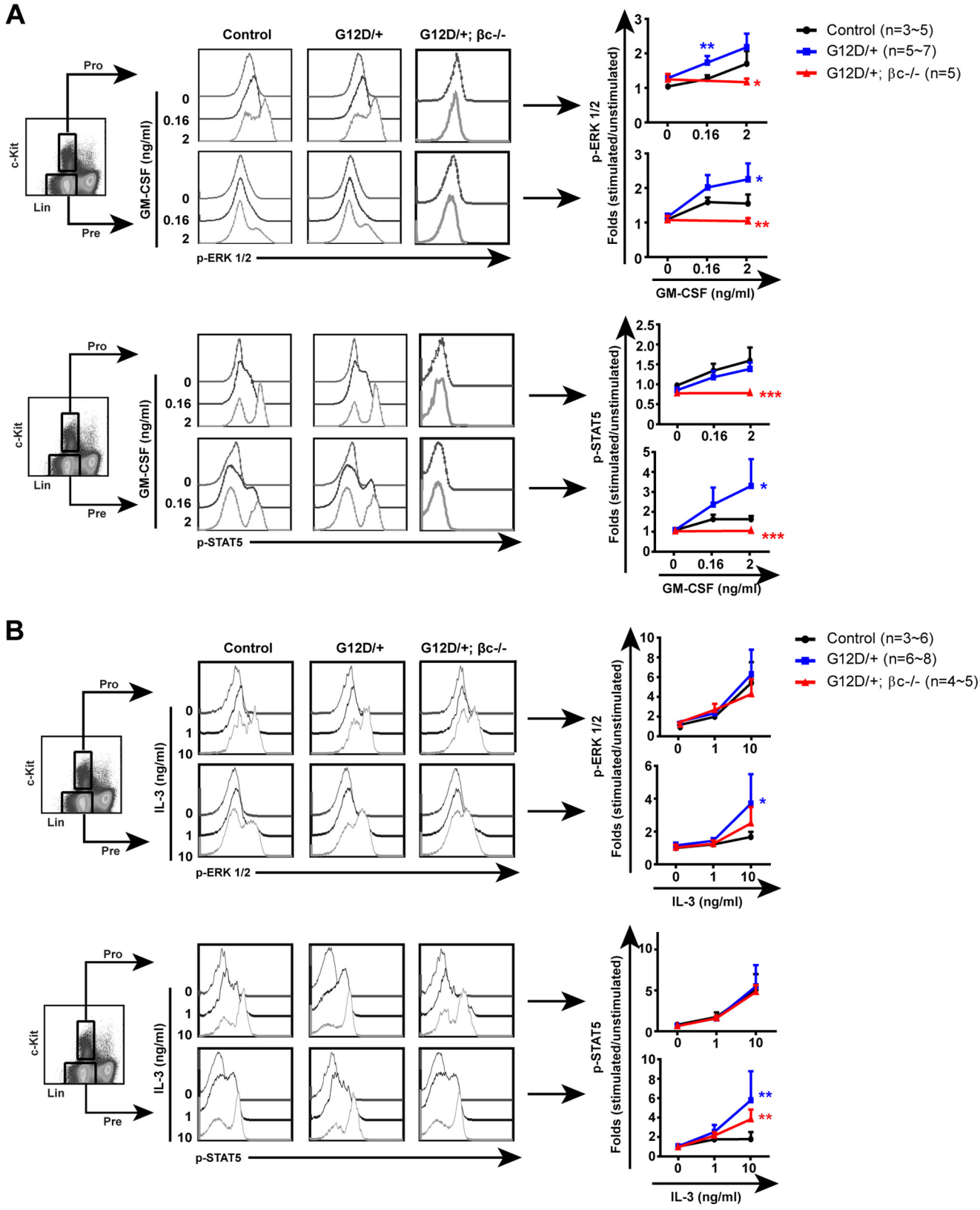


FIGURE 2. **βc deficiency abolishes GM-CSF signaling but preserves IL-3 signaling in *Nras*$^{G12D/+}$ cells.** *A* and *B*, total bone marrow cells isolated from control, *Nras*$^{G12D/+}$, or *Nras*$^{G12D/+}$; *βc*$^{-/-}$ mice on Day 12 were serum- and cytokine-starved for 2 h and stimulated with various concentrations of mGM-CSF (0, 0.16 and 2 ng/ml) (*A*) or mIL-3 (0, 1, 10 ng/ml) (*B*) at 37 °C for 10 min. Levels of p-ERK1/2 and p-Stat5 were measured using phospho–specific flow cytometry. Non-neutrophil cells (enriched for undifferentiated progenitor and precursor cells) were gated for data analysis. Myeloid progenitors are enriched in Lin$^{low/-}$ cKit^{+} cells (*Pro*), whereas myeloid precursors are enriched in Lin$^{low/-}$ cKit^{-} cells (*Pre*). Data are presented as mean ± S.D. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

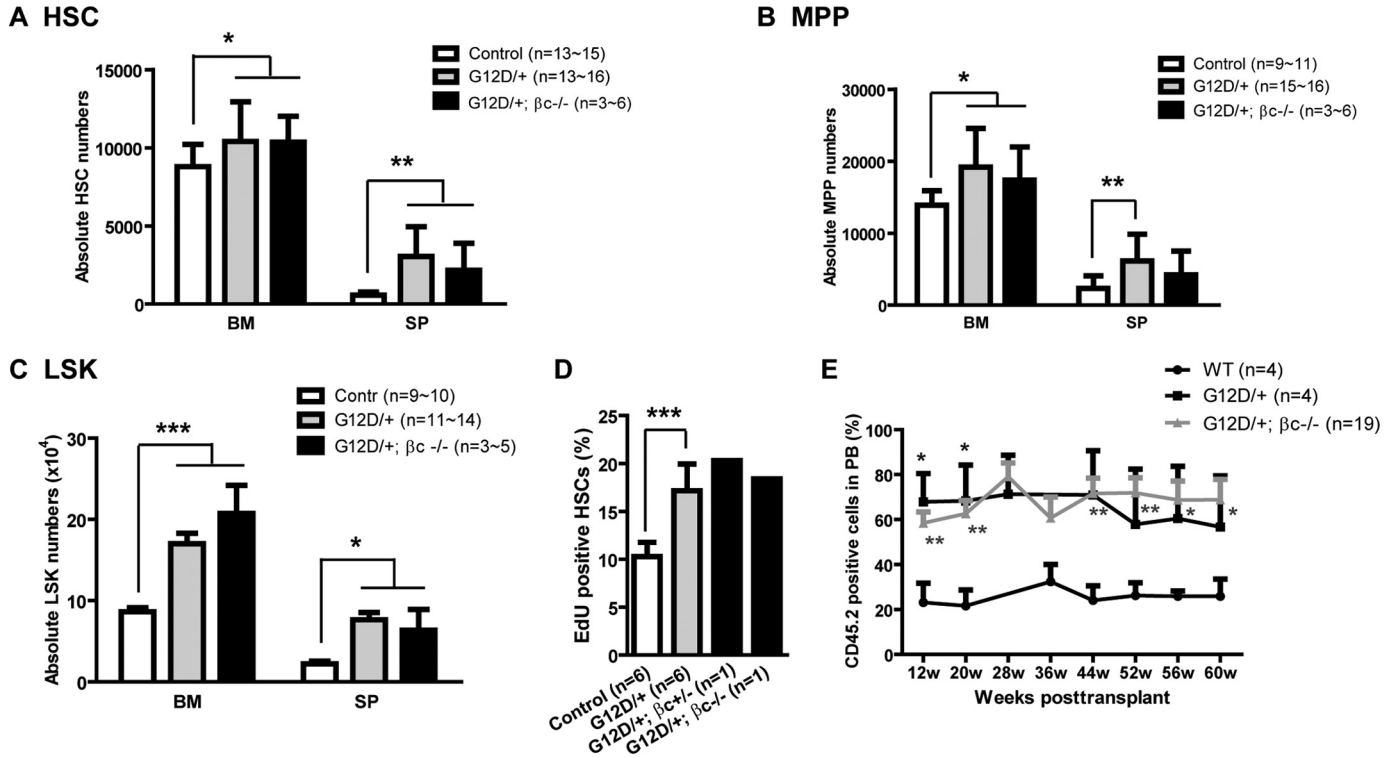


FIGURE 3. **Deletion of *βc* does not affect *Nras$^{G12D/+}$* HSCs.** Mice were treated with pI-pC and euthanized on Day 12 for analysis as described under "Materials and Methods." *A–C*, Lin$^-$ CD41$^-$ CD48$^-$ cKit$^+$ Sca1$^+$ CD150$^+$ HSCs (*A*), Lin$^-$ CD41$^-$ CD48$^-$ cKit$^+$ Sca1$^+$ CD150$^-$ multipotent progenitors (*MPPs*) (*B*), and LSK cells (*C*) from bone marrow (*BM*) and spleen (*SP*) were quantified using flow cytometry. *D*, a 16-h pulse of EdU to quantify proliferating HSCs in bone marrow. *E*, 2.5 × 10^5 bone marrow cells from control, *Nras$^{G12D/+}$*, or *Nras$^{G12D/+}$; βc$^{-/-}$* mice were transplanted with the same number of competitor cells into lethally irradiated mice. The percentages of donor-derived cells (CD45.2$^+$) in the peripheral blood of recipient mice were examined at multiple time points after transplantation. Data are presented as mean ± S.D. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Scientific). Mouse tissues were fixed in 10% neutral buffered formalin (Sigma-Aldrich) and further processed at the University of Wisconsin Carbone Comprehensive Cancer Center (UWCCC) Histology Lab.

Immunohistochemistry—Immunohistochemistry was performed on the Ventana Discovery XT biomarker platform. Sections were pretreated through antigen retrieval using CC2, a proprietary citrate buffer (Ventana, 760-107). The following steps were carried out with Reaction Buffer (Ventana 950-300) rinses between them. Primary antibody pan-cytokeratin (Thermo Scientific, PIPA521985) was diluted 1:100 using Renoir Red antibody diluent (BioCare Medical, PD904H), applied to sections, and incubated for 28 min at 37 °C. OmniMap anti-rabbit HRP biotin-free conjugate (Ventana, 760-4311) was applied for 12 min at 37 °C followed by detection using ChromoMap DAB kit (Ventana, 760-159) for 8 min. Tissues were counterstained with CAT hematoxylin (BioCare Medical, CATHE-M) and coverslipped. All these steps were performed by the Department of Pathology's Translational Research Initiatives in Pathology, part of the UWCCC Translational Science BioCore.

Results

Deletion of βc Decreases Oncogenic Nras-induced Splenomegaly—To investigate whether deletion of *βc* affects the hematopoietic phenotypes induced by oncogenic Nras, we generated *Mx1-Cre*, *Nras$^{LSL\ G12D/+}$; Mx1-Cre* and *Nras$^{LSL\ G12D/+}$; Mx1-Cre; βc$^{-/-}$* mice (Fig. 1, *A* and *B*). At 5–7 weeks of age, these mice were administrated with pI-pC, which stimulates endogenous interferon production and induces Cre expression in various tissues but predominantly the hematopoietic tissues from the interferon-inducible promoter Mx1 (32). The Cre recombinase subsequently removed the stop cassette and induced oncogenic Nras expression from its endogenous locus. The day of the first pI-pC injection is defined as Day 1. After two rounds of pI-pC injection, all mice were sacrificed on Day 12. We refer to the pI-pC-treated compound mice as *Nras$^{G12D/+}$* and *Nras$^{G12D/+}$; βc$^{-/-}$* mice, respectively, and pI-pC-treated *Mx1-Cre* mice as control mice throughout this study.[3]

After acute induction of oncogenic Nras expression in hematopoietic tissues, both *Nras$^{G12D/+}$* and *Nras$^{G12D/+}$; βc$^{-/-}$* mice were grossly normal, with unremarkable white blood cell counts, hematocrit, and platelet counts (Fig. 1*D*) and normal myeloid differentiation in bone marrow and peripheral blood (Fig. 1*E*). However, *Nras$^{G12D/+}$* mice showed moderately but significantly enlarged spleen when compared with control mice (Fig. 1*C*). Flow cytometric analysis demonstrated that the percentages of granulocytes (Mac$^+$ Gr1$^+$) and monocytes (Mac1$^+$ Gr1$^-$) were significantly increased in *Nras$^{G12D/+}$* spleens when

[3] Mouse genotype abbreviations are: control, pI-pC-treated Mx1-Cre; Nras$^{G12D/+}$, recombined Nras$^{G12D/+}$ heterozygous (pI-pC-treated Nras$^{LSL\ G12D/+}$; Mx1-Cre); Nras$^{G12D/+}$; βc$^{-/-}$, recombined Nras$^{G12D/+}$ heterozygous with germ line deletion of βc (pI-pC-treated Nras$^{LSL\ G12D/+}$; Mx1-Cre; βc$^{-/-}$).

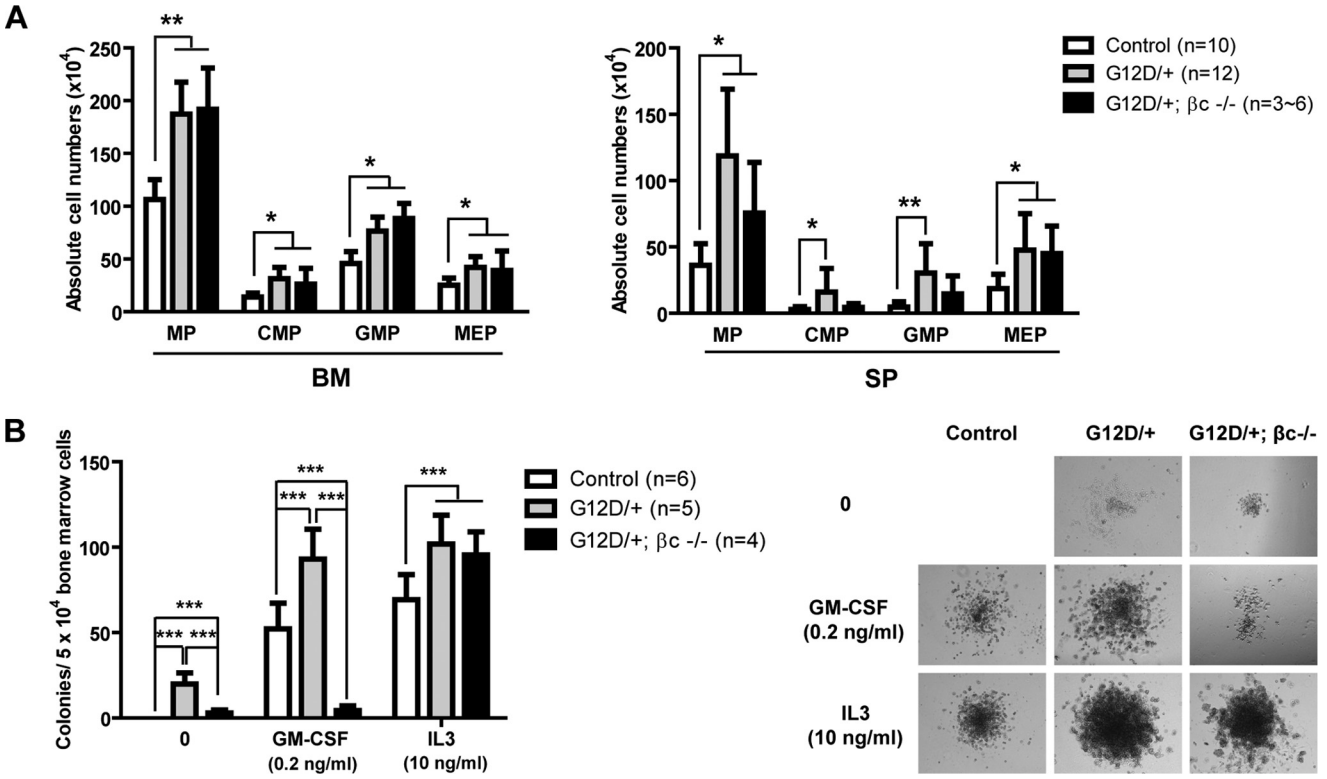


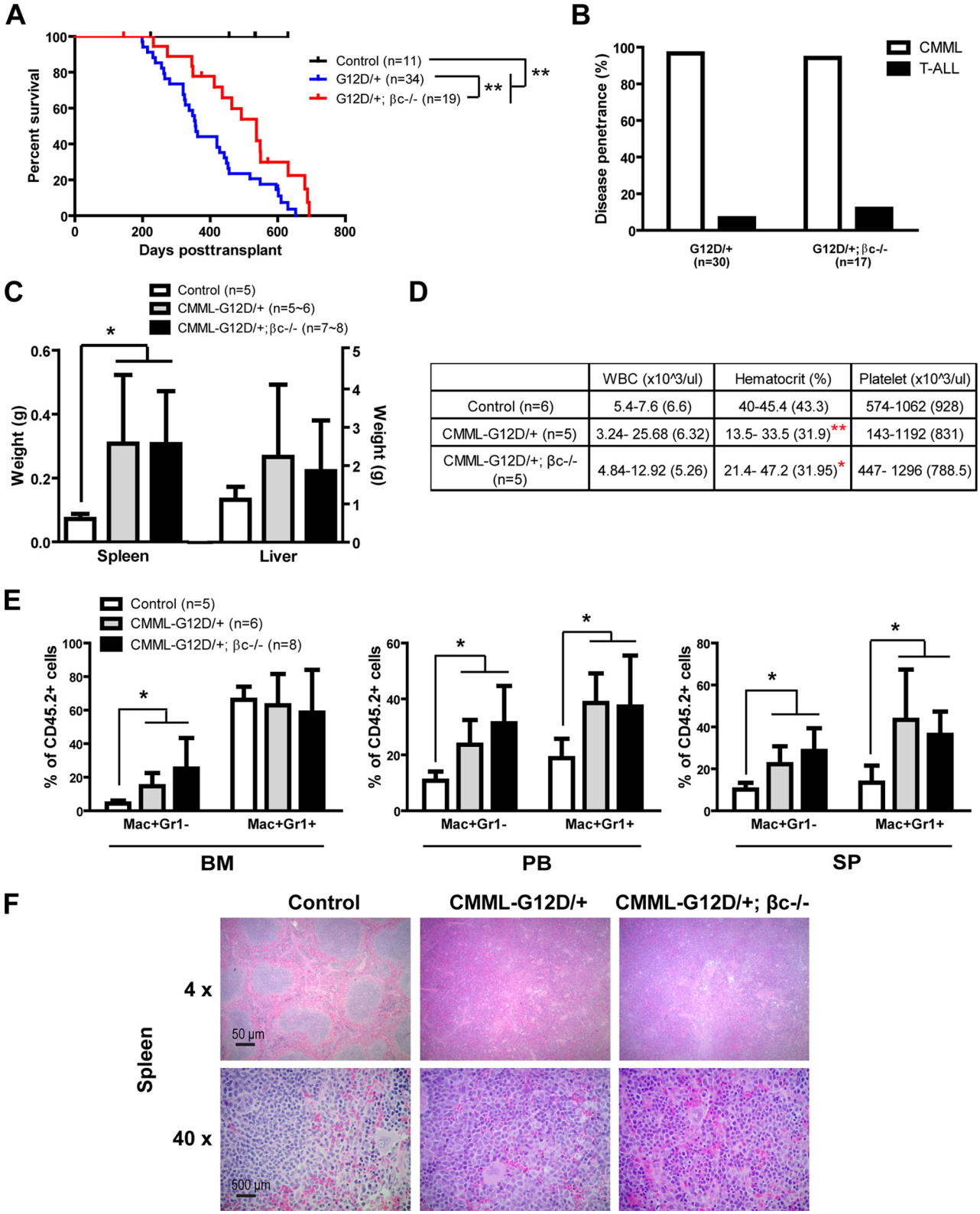
FIGURE 4. **Loss of βc greatly reduces $Nras^{G12D/+}$-induced spontaneous colony formation.** Mice were treated with pI-pC and euthanized on Day 12 for analysis as described under "Materials and Methods." *A*, quantification of MPs in bone marrow (*BM*) and spleen (*SP*). *CMP*, common myeloid progenitor; *GMP*, granulocyte-monocyte progenitor; *MEP*, megakaryocyte-erythroid progenitors. *B*, 5×10^4 bone marrow cells isolated from control, $Nras^{G12D/+}$, or $Nras^{G12D/+}$; $\beta c^{-/-}$ mice were plated in semi-solid medium without cytokine or with 0.2 ng/ml mGM-CSF or 10 ng/ml mIL-3. Colonies were counted 7–10 days after culture. Data are presented as mean ± S.D. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

compared with those in control spleens (Fig. 1*E*). Noticeably, the splenomegaly and percentage of splenic monocytes were significantly reduced in $Nras^{G12D/+}$; $\beta c^{-/-}$ mice when compared with those in $Nras^{G12D/+}$ mice (Fig. 1, *C* and *E*). These results indicate that deletion of βc decreases oncogenic *Nras*-induced monocytic cell expansion in spleen, which might contribute to the reduced splenomegaly in $Nras^{G12D/+}$; $\beta c^{-/-}$ mice.

Loss of βc Abolishes GM-CSF Signaling but Preserves IL-3 Signaling in $Nras^{G12D/+}$ Cells—To determine whether loss of βc affects cytokine signaling in $Nras^{G12D/+}$ cells, we studied GM-CSF signaling and IL-3 signaling in $Lin^{low/-}$ $cKit^+$ cells (enriched for MPs, early progenitor cells that give rise to various types of myeloid cells) and $Lin^{low/-}$ $cKit^-$ cells (enriched for myeloid precursors, immature myeloid cells that are downstream of MPs) from control, $Nras^{G12D/+}$, and $Nras^{G12D/+}$; $\beta c^{-/-}$ mice (Fig. 2). We found that GM-CSF- and IL-3-evoked ERK1/2 and STAT5 activation in $Nras^{G12D/+}$ MPs was largely comparable with that in control cells, whereas $Nras^{G12D/+}$ myeloid precursors showed moderate but significant hyperactivation upon stimulation with saturated concentrations of cytokines. In the absence of βc, GM-CSF signaling was completely abolished in $Nras^{G12D/+}$; $\beta c^{-/-}$ cells (Fig. 2*A*), whereas IL-3 signaling remained intact (Fig. 2*B*).

βc Is Dispensable for $Nras^{G12D/+}$ HSCs—Because $Nras^{G12D/+}$ HSCs serve as CMML-initiating cells (27), we investigated whether deletion of βc affects their functions in leukemogenesis. We first examined the HSC compartment in control, $Nras^{G12D/+}$, and $Nras^{G12D/+}$; $\beta c^{-/-}$ mice on Day 12. HSCs were defined as Lin^- $CD41^-$ $CD48^-$ $cKit^+$ $Sca1^+$ $CD150^+$ cells (33, 34). The absolute HSC numbers in bone marrow and spleen of $Nras^{G12D/+}$; $\beta c^{-/-}$ mice were moderately but significantly increased when compared with those in control mice but comparable with those in $Nras^{G12D/+}$ mice (Fig. 3*A*). Concomitantly, the compartments of multipotent progenitors (Lin^- $CD41^-$ $CD48^-$ $cKit^+$ $Sca1^+$ $CD150^-$) (33) and LSK cells in bone marrow and/or spleen of $Nras^{G12D/+}$; $\beta c^{-/-}$ mice were also expanded but indistinguishable from those in $Nras^{G12D/+}$ mice (Fig. 3, *B* and *C*). These results indicate that deletion of βc does not affect oncogenic *Nras*-induced HSC expansion.

To investigate whether deletion of βc affects increased proliferation of $Nras^{G12D/+}$ HSCs (27), we performed EdU incorporation analysis. The percentages of cycling $Nras^{G12D/+}$; $\beta c^{+/-}$ and $Nras^{G12D/+}$; $\beta c^{-/-}$ HSCs seemed to be higher than control HSCs but comparable with $Nras^{G12D/+}$ HSCs (Fig. 3*D*). To investigate further whether deletion of βc affects increased self-renewal of $Nras^{G12D/+}$ HSCs (27), we transplanted 2.5×10^5 total bone marrow cells ($CD45.2^+$) isolated from control, $Nras^{G12D/+}$, and $Nras^{G12D/+}$; $\beta c^{-/-}$ mice, accompanied by the same number of congenic competitors ($CD45.1^+$) into lethally irradiated recipient mice ($CD45.1^+$). We found that over 1 year of time, donor-derived blood cells in recipients transplanted with $Nras^{G12D/+}$; $\beta c^{-/-}$ cells were stably maintained at a much higher reconstitution rate when compared with those in recipients with control cells but at a similar level as recipients with $Nras^{G12D/+}$ cells (Fig. 3*E*). These data



	WBC (x10^3/ul)	Hematocrit (%)	Platelet (x10^3/ul)
Control (n=6)	5.4-7.6 (6.6)	40-45.4 (43.3)	574-1062 (928)
CMML-G12D/+ (n=5)	3.24- 25.68 (6.32)	13.5- 33.5 (31.9)**	143-1192 (831)
CMML-G12D/+; βc-/- (n=5)	4.84-12.92 (5.26)	21.4- 47.2 (31.95)*	447- 1296 (788.5)

FIGURE 5. **Deletion of *βc* slows down the progression of *Nras*$^{G12D/+}$-induced hematopoietic malignancies.** Lethally irradiated mice were transplanted with 2.5 × 10^5 total bone marrow cells from control, *Nras*$^{G12D/+}$, or *Nras*$^{G12D/+}$; *βc*$^{-/-}$ mice along with the same number of competitor cells. *A*, Kaplan-Meier comparative survival analysis of reconstituted mice. Cumulative survival was plotted against days after transplantation. *p* value was determined by the log-rank test. *B*, disease incidence in recipient mice transplanted with *Nras*$^{G12D/+}$ or *Nras*$^{G12D/+}$; *βc*$^{-/-}$ cells. *T-ALL*, T-cell acute lymphoblastic leukemia/lymphoma. *C*, spleen and liver weight of moribund CMML-*Nras*$^{G12D/+}$ or CMML-*Nras*$^{G12D/+}$; *βc*$^{-/-}$ mice. *D*, complete blood count was performed on peripheral blood samples collected from moribund CMML-*Nras*$^{G12D/+}$ or CMML-*Nras*$^{G12D/+}$; *βc*$^{-/-}$ mice and age-matched control mice. *E*, flow cytometry analysis of bone marrow (*BM*), peripheral blood (*PB*), and spleen (*SP*) cells from control and moribund CMML-*Nras*$^{G12D/+}$ and CMML-*Nras*$^{G12D/+}$; *βc*$^{-/-}$ mice using myeloid lineage-specific markers. *F*, representative spleen histologic H&E sections from moribund CMML-*Nras*$^{G12D/+}$ and CMML-*Nras*$^{G12D/+}$; *βc*$^{-/-}$ mice and age-matched control mice. Data are presented as mean ± S.D. *, $p < 0.05$; **, $p < 0.01$.

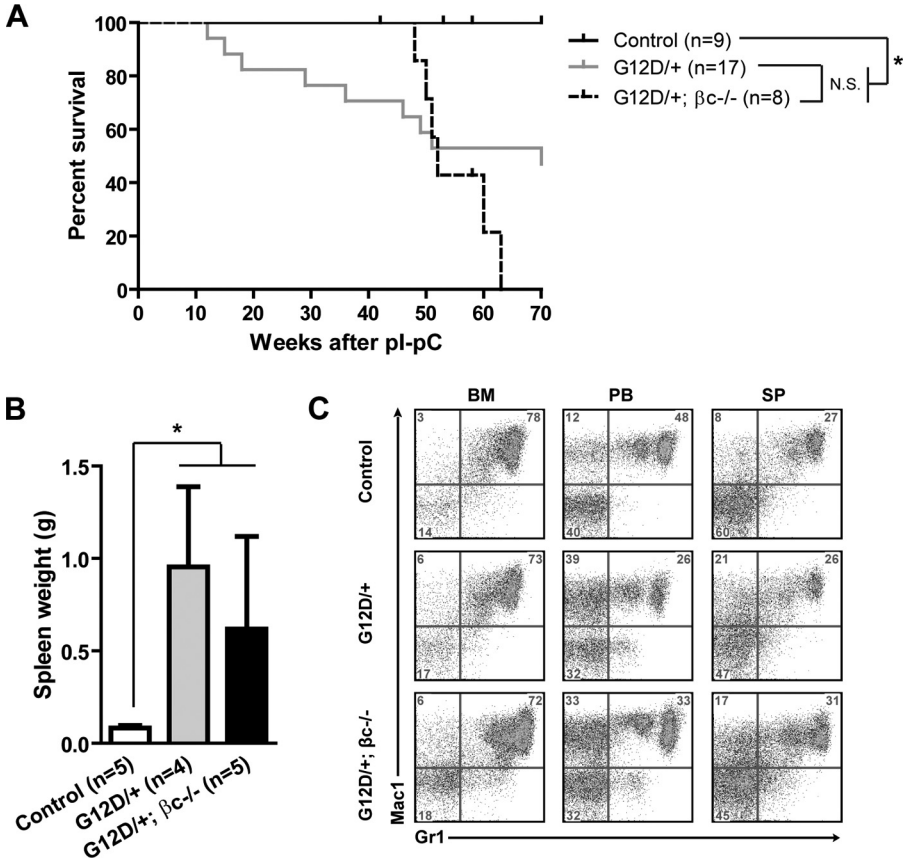


FIGURE 6. ***$Nras^{G12D/+}$; $\beta c^{-/-}$* mice develop CMML-like phenotypes after a prolonged latency.** *A*, Kaplan-Meier comparative survival analysis of non-transplanted control, $Nras^{G12D/+}$, and $Nras^{G12D/+}$; $\beta c^{-/-}$ mice. Cumulative survival was plotted against weeks after the last pI-pC injection. *p* value was determined by the log-rank test. *B*, spleen weights of moribund $Nras^{G12D/+}$ and $Nras^{G12D/+}$; $\beta c^{-/-}$ mice and age-matched control mice. Data are presented as mean ± S.D. *C*, representative flow analysis of myeloid cells from moribund $Nras^{G12D/+}$ and $Nras^{G12D/+}$; $\beta c^{-/-}$ mice and age-matched control mice. *N.S.*, not significant; *, $p < 0.05$.

suggest that deletion of *βc* does not affect increased self-renewal of $Nras^{G12D/+}$ HSCs. Together, our results demonstrate that βc is dispensable for $Nras^{G12D/+}$ HSCs.

Loss of βc Abolishes Oncogenic Nras-induced Spontaneous Colony Formation—To test whether loss of *βc* affects MP expansion in $Nras^{G12D/+}$ mice, we analyzed the MP (Lin^- $IL7R\alpha^-$ $Sca1^-$ $cKit^+$) compartment in control, $Nras^{G12D/+}$, and $Nras^{G12D/+}$; $\beta c^{-/-}$ mice. The absolute numbers of MPs, including common myeloid progenitors, granulocyte-monocyte progenitors, and megakaryocyte-erythroid progenitors, in $Nras^{G12D/+}$ and $Nras^{G12D/+}$; $\beta c^{-/-}$ bone marrow were comparable with each other, and both numbers were significantly higher than those in control bone marrow (Fig. 4*A*). A similar trend was also observed in spleen (Fig. 4*A*). Our data indicate that deletion of *βc* does not affect oncogenic *Nras*-induced MP expansion.

We and others previously reported that bone marrow cells from $Nras^{G12D/+}$ mice form a significant number of colonies in the absence of cytokines (10, 11). To determine whether the spontaneous colony formation of $Nras^{G12D/+}$ cells depends on βc-mediated signaling, we isolated bone marrow cells from control, $Nras^{G12D/+}$, and $Nras^{G12D/+}$; $\beta c^{-/-}$ mice and plated them in semi-solid medium in the absence of cytokines. Consistent with previous studies (10, 11), $Nras^{G12D/+}$ cells formed a significant number of colonies, whereas $Nras^{G12D/+}$; $\beta c^{-/-}$ cells formed a much lower number of colonies (Fig. 4*B*). In the presence of mGM-CSF or mIL-3, $Nras^{G12D/+}$ cells formed significantly more and bigger colonies than control cells (Fig. 4*B*). As expected, $Nras^{G12D/+}$; $\beta c^{-/-}$ cells did not form a significant number of colonies in the presence of mGM-CSF but formed a comparable number and size of colonies as $Nras^{G12D/+}$ cells in the presence of mIL-3, consistent with our signaling studies (Fig. 4*B*). Our results suggest that the spontaneous colony formation of $Nras^{G12D/+}$ cells largely depends on βc-mediated GM-CSF signaling.

βc Deficiency Significantly Delays the Progression of Oncogenic Nras-induced Leukemias in a Cell-autonomous Manner—To investigate whether *βc* deficiency attenuates oncogenic *Nras*-induced leukemogenesis in a hematopoietic cell-specific manner, we transplanted bone marrow cells from control, $Nras^{G12D/+}$, and $Nras^{G12D/+}$; $\beta c^{-/-}$ mice together with competitor cells into lethally irradiated mice (Fig. 5). Consistent with our previous study (10), ~97% of recipients transplanted with $Nras^{G12D/+}$ cells developed a CMML-like disease and ~7% developed acute T-cell lymphoblastic leukemia/lymphoma. Similarly, ~94% of recipients with $Nras^{G12D/+}$; $\beta c^{-/-}$ cells developed a CMML-like disease and ~12% developed T-cell lymphoblastic leukemia/lymphoma (Fig. 5*B*). Some mice developed both diseases. However, recipients transplanted with $Nras^{G12D/+}$; $\beta c^{-/-}$ cells survived significantly longer than

those with *Nras*$^{G12D/+}$ cells (median survival: 537 days *versus* 357 days) (Fig. 5*A*). Despite different survival curves, both groups of mice with CMML displayed similar disease phenotypes at the moribund stage, including markedly enlarged spleen (Fig. 5*C*) with significant extramedullary hematopoiesis (Fig. 5*F*), increased white blood cell counts and anemia (Fig. 5*D*), and a predominant expansion of granulocytes and/or monocytes in hematopoietic tissues (Fig. 5*E*). These results indicate that deletion of *βc* cannot abrogate oncogenic *Nras*-induced CMML formation but it does significantly delay CMML progression.

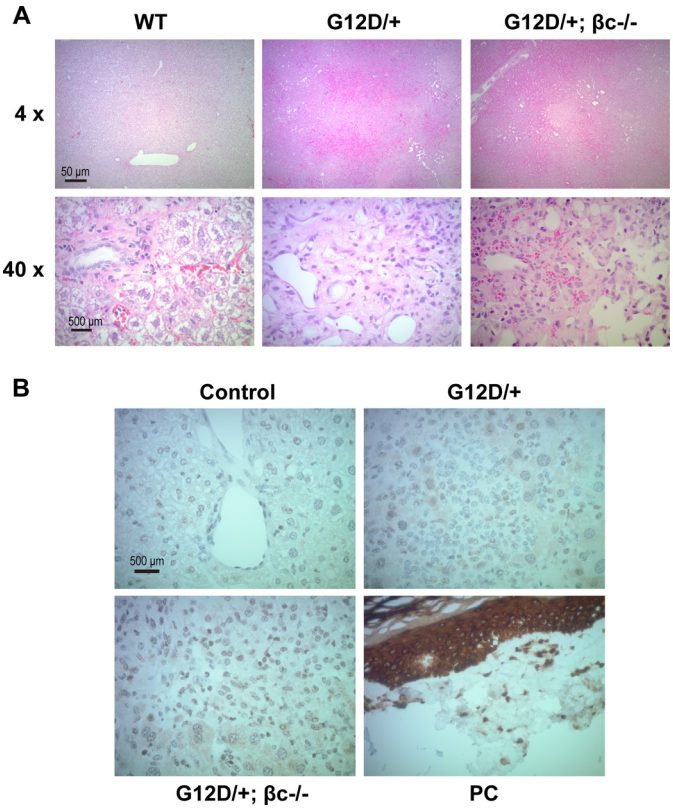


FIGURE 7. **Analysis of livers from moribund *Nras*$^{G12D/+}$ and *Nras*$^{G12D/+}$; *βc*$^{-/-}$ mice and age-matched control mice.** *A*, representative liver histologic H&E sections. *B*, pan-cytokeratin staining in liver. Skin was used as a positive control (*PC*) for keratin staining.

Deletion of βc in Nras$^{G12D/+}$ *Mice Promotes Hepatic Histiocytic Sarcomas with Atypical Morphology*—Our previous results show that ~50% of primary *Nras*$^{G12D/+}$ mice (7 out of 15) died with hepatic histiocytic sarcoma within a year after pI-pC injections (10). Although the median survival of *Nras*$^{G12D/+}$; *βc*$^{-/-}$ mice was indistinguishable from that of *Nras*$^{G12D/+}$ mice, the disease latency was prolonged (Fig. 6). Like *Nras*$^{G12D/+}$ mice, most of the *Nras*$^{G12D/+}$; *βc*$^{-/-}$ mice (6 out of 8) developed CMML-like phenotypes with increased monocytosis in peripheral blood (Fig. 6*C*). Two out of three *Nras*$^{G12D/+}$; *βc*$^{-/-}$ mice also developed multiple hepatic tumor nodules with varying morphology but consisting of histiocyte-like cells ranging from small and monotonous to large and multinucleated. Because the tumors also entrapped steatotic hepatocytes and residual sinusoidal endothelial channels (Fig. 7*A*), we initially considered whether this might represent a non-hematopoietic tumor type, but negative pan-keratin staining (Fig. 7*B*) and the overall morphologic features favor a histiocytic/monocytic derived neoplasm. We speculate that the morphologic differences between these tumors and those we have previously described in *Nras*$^{G12D/+}$ mice (10) might be due to their inability to normally respond to GM-CSF-derived signal.

Discussion

In this study, we show that *βc* deficiency indeed abolishes GM-CSF signaling in *Nras*$^{G12D/+}$ cells but IL-3 signaling is preserved (Fig. 2). Consequently, loss of *βc* does not affect *Nras*$^{G12D/+}$ HSC functions (Fig. 3) and therefore does not abrogate CMML in *Nras*$^{G12D/+}$ mice (Fig. 5). However, deletion of *βc* does significantly slow down the progression of CMML and prolong the survival of recipients transplanted with *Nras*$^{G12D/+}$ cells (Fig. 5). Our findings are summarized in Fig. 8.

We previously reported that in the *Nras*$^{G12D/+}$-induced CMML model, mutant HSCs are required to initiate and maintain CMML-like phenotypes and serve as CMML-initiating cells (27). Consistent with an earlier report that *βc* is dispensable for normal HSCs (35), we found that *βc* is dispensable for *Nras*$^{G12D/+}$ HSCs as well; loss of *βc* does not affect the expansion, increased proliferation and self-renewal, and myeloid differentiation bias in *Nras*$^{G12D/+}$ HSCs (Figs. 3 and 4). We believe that *βc* is also dispensable for *Nf1*$^{-/-}$ HSCs. Therefore, it is not surprising that the MP compartment remains expanded in

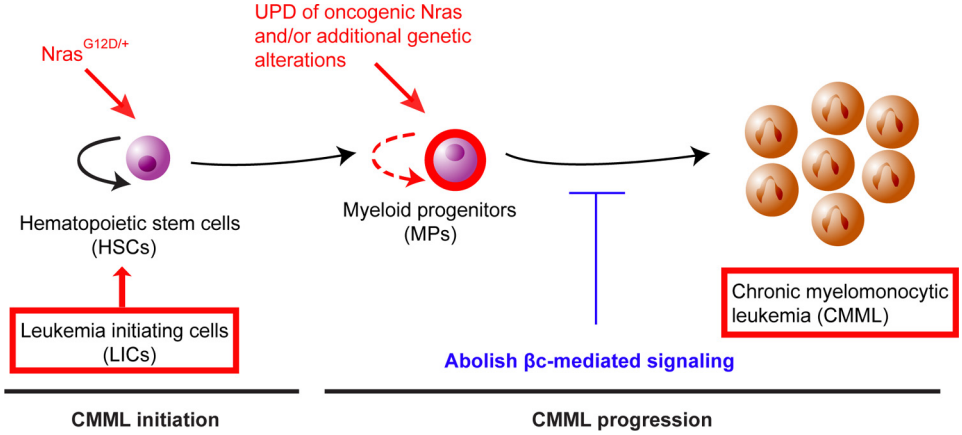


FIGURE 8. **Schematic picture illustrating the role of *βc*-mediated signaling in *Nras*$^{G12D/+}$-induced CMML.** *UPD*, uniparental disomy.

$Nras^{G12D/+}$; $\beta c^{-/-}$ (Fig. 4) and $Nf1^{-/-}$; $\beta c^{-/-}$ mice and that deletion of βc does not abrogate CMML in these animals (26).

Despite potential redundancy of other cytokine signaling (*e.g.* IL-3, G-CSF, and M-CSF) in the absence of βc-mediated GM-CSF signaling, βc deficiency indeed significantly reduces $Nras^{G12D/+}$-induced splenomegaly (Fig. 1*C*) and spontaneous colony formation (Fig. 4*B*) and prolongs the survival of CMML mice (Fig. 5*A*), suggesting that GM-CSF signaling plays an important role in promoting CMML progression. Our result is consistent with previous human and mouse studies (10, 21–23, 36). However, in t(8;21)-induced acute myeloid leukemia (AML), GM-CSF is found to reduce the replating ability of RUNX1-ETO-expressing cells and therefore has a negative impact on leukemogenesis; expression of RUNX1-ETO in $\beta c^{-/-}$ cells leads to a high penetrance of AML (37). Therefore, hyperactive GM-CSF signaling potentially opposes AML formation by inhibiting transformation of MPs to AML-initiating cells. This might explain the absence of spontaneous AML in oncogenic Ras models and the low incidence of transformation to AML in JMML patients. Although we and others did not see AML genesis in $Nras^{G12D/+}$; $\beta c^{-/-}$ and $Nf1^{-/-}$; $\beta c^{-/-}$ mice, we could not rule out the possibility that long-term inhibition of GM-CSF signaling in JMML/CMML patients might increase their risk to develop AML.

Author Contributions—J. F. Z and J. Z. conceived the idea. J. F. Z, J. D., Y. L., J. W., and G. K. performed the experiments. J. F. Z, E. A. R., and J. Z. analyzed and interpreted the data. J. F. Z, E. A. R., and J. Z. wrote the manuscript.

Acknowledgments—We thank the University of Wisconsin Carbone Comprehensive Cancer Center (UWCCC) for use of its Shared Services to complete this research.

References

1. Emanuel, P. D. (2008) Juvenile myelomonocytic leukemia and chronic myelomonocytic leukemia. *Leukemia* **22,** 1335–1342
2. Elliott, M. A. (2006) Chronic neutrophilic leukemia and chronic myelomonocytic leukemia: WHO defined. *Best Pract. Res. Clin. Haematol.* **19,** 571–593
3. Tartaglia, M., Niemeyer, C. M., Fragale, A., Song, X., Buechner, J., Jung, A., Hählen, K., Hasle, H., Licht, J. D., and Gelb, B. D. (2003) Somatic mutations in *PTPN11* in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. *Nat. Genet.* **34,** 148–150
4. Yoshida, N., Yagasaki, H., Xu, Y., Matsuda, K., Yoshimi, A., Takahashi, Y., Hama, A., Nishio, N., Muramatsu, H., Watanabe, N., Matsumoto, K., Kato, K., Ueyama, J., Inada, H., Goto, H., Yabe, M., Kudo, K., Mimaya, J., Kikuchi, A., Manabe, A., Koike, K., and Kojima, S. (2009) Correlation of clinical features with the mutational status of GM-CSF signaling pathway-related genes in juvenile myelomonocytic leukemia. *Pediatr. Res.* **65,** 334–340
5. Loh, M. L., Sakai, D. S., Flotho, C., Kang, M., Fliegauf, M., Archambeault, S., Mullighan, C. G., Chen, L., Bergstraesser, E., Bueso-Ramos, C. E., Emanuel, P. D., Hasle, H., Issa, J. P., van den Heuvel-Eibrink, M. M., Locatelli, F., Stary, J., Trebo, M., Wlodarski, M., Zecca, M., Shannon, K. M., and Niemeyer, C. M. (2009) Mutations in *CBL* occur frequently in juvenile myelomonocytic leukemia. *Blood* **114,** 1859–1863
6. Onida, F., and Beran, M. (2004) Chronic myelomonocytic leukemia: myeloproliferative variant. *Curr. Hematol. Rep.* **3,** 218–226
7. Chan, I. T., Kutok, J. L., Williams, I. R., Cohen, S., Kelly, L., Shigematsu, H., Johnson, L., Akashi, K., Tuveson, D. A., Jacks, T., and Gilliland, D. G. (2004) Conditional expression of oncogenic *K-ras* from its endogenous promoter induces a myeloproliferative disease. *J. Clin. Invest.* **113,** 528–538
8. Braun, B. S., Tuveson, D. A., Kong, N., Le, D. T., Kogan, S. C., Rozmus, J., Le Beau, M. M., Jacks, T. E., and Shannon, K. M. (2004) Somatic activation of oncogenic *Kras* in hematopoietic cells initiates a rapidly fatal myeloproliferative disorder. *Proc. Natl. Acad. Sci. U.S.A.* **101,** 597–602
9. Zhang, J., Wang, J., Liu, Y., Sidik, H., Young, K. H., Lodish, H. F., and Fleming, M. D. (2009) Oncogenic *Kras*-induced leukemogeneis: hematopoietic stem cells as the initial target and lineage-specific progenitors as the potential targets for final leukemic transformation. *Blood* **113,** 1304–1314
10. Wang, J., Liu, Y., Li, Z., Du, J., Ryu, M. J., Taylor, P. R., Fleming, M. D., Young, K. H., Pitot, H., and Zhang, J. (2010) Endogenous oncogenic *Nras* mutation leads to aberrant GM-CSF signaling in granulocytic/monocytic precursors in a murine model of chronic myelomonocytic leukemia. *Blood* **116,** 5991–6002
11. Li, Q., Haigis, K. M., McDaniel, A., Harding-Theobald, E., Kogan, S. C., Akagi, K., Wong, J. C., Braun, B. S., Wolff, L., Jacks, T., and Shannon, K. (2011) Hematopoiesis and leukemogenesis in mice expressing oncogenic $Nras^{G12D}$ from the endogenous locus. *Blood* **117,** 2022–2032
12. Wang, J., Liu, Y., Li, Z., Wang, Z., Tan, L. X., Ryu, M. J., Meline, B., Du, J., Young, K. H., Ranheim, E., Chang, Q., and Zhang, J. (2011) Endogenous oncogenic *Nras* mutation initiates hematopoietic malignancies in a dose- and cell type-dependent manner. *Blood* **118,** 368–379
13. Largaespada, D. A., Brannan, C. I., Jenkins, N. A., and Copeland, N. G. (1996) *Nf1* deficiency causes Ras-mediated granulocyte/macrophage colony stimulating factor hypersensitivity and chronic myeloid leukaemia. *Nat. Genet.* **12,** 137–143
14. Bollag, G., Clapp, D. W., Shih, S., Adler, F., Zhang, Y. Y., Thompson, P., Lange, B. J., Freedman, M. H., McCormick, F., Jacks, T., and Shannon, K. (1996) Loss of *NF1* results in activation of the Ras signaling pathway and leads to aberrant growth in haematopoietic cells. *Nat. Genet.* **12,** 144–148
15. Le, D. T., Kong, N., Zhu, Y., Lauchle, J. O., Aiyigari, A., Braun, B. S., Wang, E., Kogan, S. C., Le Beau, M. M., Parada, L., and Shannon, K. M. (2004) Somatic inactivation of *Nf1* in hematopoietic cells results in a progressive myeloproliferative disorder. *Blood* **103,** 4243–4250
16. Emanuel, P. D., Bates, L. J., Castleberry, R. P., Gualtieri, R. J., and Zuckerman, K. S. (1991) Selective hypersensitivity to granulocyte-macrophage colony-stimulating factor by juvenile chronic myeloid leukemia hematopoietic progenitors. *Blood* **77,** 925–929
17. Bowen, D. T. (2005) Chronic myelomonocytic leukemia: lost in classification? *Hematol. Oncol.* **23,** 26–33
18. Cambier, N., Baruchel, A., Schlageter, M. H., Menot, M. L., Wattel, E., Fenaux, P., and Chomienne, C. (1997) Chronic myelomonocytic leukemia: from biology to therapy. *Hematol. Cell Ther.* **39,** 41–48
19. Hercus, T. R., Thomas, D., Guthridge, M. A., Ekert, P. G., King-Scott, J., Parker, M. W., and Lopez, A. F. (2009) The granulocyte-macrophage colony-stimulating factor receptor: linking its structure to cell signaling and its role in disease. *Blood* **114,** 1289–1298
20. Shi, Y., Liu, C. H., Roberts, A. I., Das, J., Xu, G., Ren, G., Zhang, Y., Zhang, L., Yuan, Z. R., Tan, H. S., Das, G., and Devadas, S. (2006) Granulocyte-macrophage colony-stimulating factor (GM-CSF) and T-cell responses: what we do and don't know. *Cell Res.* **16,** 126–133
21. Kotecha, N., Flores, N. J., Irish, J. M., Simonds, E. F., Sakai, D. S., Archambeault, S., Diaz-Flores, E., Coram, M., Shannon, K. M., Nolan, G. P., and Loh, M. L. (2008) Single-cell profiling identifies aberrant STAT5 activation in myeloid malignancies with specific clinical and biologic correlates. *Cancer Cell* **14,** 335–343
22. Iversen, P. O., Lewis, I. D., Turczynowicz, S., Hasle, H., Niemeyer, C., Schmiegelow, K., Bastiras, S., Biondi, A., Hughes, T. P., and Lopez, A. F. (1997) Inhibition of granulocyte-macrophage colony-stimulating factor prevents dissemination and induces remission of juvenile myelomonocytic leukemia in engrafted immunodeficient mice. *Blood* **90,** 4910–4917
23. Bernard, F., Thomas, C., Emile, J. F., Hercus, T., Cassinat, B., Chomienne, C., and Donadieu, J. (2002) Transient hematologic and clinical effect of E21R in a child with end-stage juvenile myelomonocytic leukemia. *Blood* **99,** 2615–2616
24. Birnbaum, R. A., O'Marcaigh, A., Wardak, Z., Zhang, Y. Y., Dranoff, G.,

Jacks, T., Clapp, D. W., and Shannon, K. M. (2000) *Nf1* and *Gmcsf* interact in myeloid leukemogenesis. *Mol. Cell* **5,** 189–195
25. Woodcock, J. M., McClure, B. J., Stomski, F. C., Elliott, M. J., Bagley, C. J., and Lopez, A. F. (1997) The human granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor exists as a preformed receptor complex that can be activated by GM-CSF, interleukin-3, or interleukin-5. *Blood* **90,** 3005–3017
26. Kim, A., Morgan, K., Hasz, D. E., Wiesner, S. M., Lauchle, J. O., Geurts, J. L., Diers, M. D., Le, D. T., Kogan, S. C., Parada, L. F., Shannon, K., and Largaespada, D. A. (2007) β common receptor inactivation attenuates myeloproliferative disease in *Nf1* mutant mice. *Blood* **109,** 1687–1691
27. Wang, J., Kong, G., Liu, Y., Du, J., Chang, Y.-I., Tey, S.R., Zhang, X., Ranheim, E. A., Saba-El-Leil, M. K., Meloche, S., Damnernsawad, A., Zhang, J., and Zhang, J. (2013) Nras$^{G12D/+}$ promotes leukemogenesis by aberrantly regulating haematopoietic stem cell functions. *Blood* **121,** 5203–5207
28. Li, Q., Bohin, N., Wen, T., Ng, V., Magee, J., Chen, S. C., Shannon, K., and Morrison, S. J. (2013) Oncogenic *Nras* has bimodal effects on stem cells that sustainably increase competitiveness. *Nature* **504,** 143–147
29. Nicola, N. A., Smith, A., Robb, L., Metcalf, D., and Begley, C. G. (1997) The structural basis of the biological actions of the GM-CSF receptor. *Ciba Found. Symp.* **204,** 19–27; discussion 27–32
30. Okuda, K., Foster, R., and Griffin, J. D. (1999) Signaling domains of the βc chain of the GM-CSF/IL-3/IL-5 receptor. *Ann. N.Y. Acad. Sci.* **872,** 305–312; discussion 312–303
31. Kong, G., Wunderlich, M., Yang, D., Ranheim, E. A., Young, K. H., Wang, J., Chang, Y. I., Du, J., Liu, Y., Tey, S. R., Zhang, X., Juckett, M., Mattison, R., Damnernsawad, A., Zhang, J., Mulloy, J. C., and Zhang, J. (2014) Combined MEK and JAK inhibition abrogates murine myeloproliferative neoplasm. *J. Clin. Invest.* **124,** 2762–2773
32. Kühn, R., Schwenk, F., Aguet, M., and Rajewsky, K. (1995) Inducible gene targeting in mice. *Science* **269,** 1427–1429
33. Kiel, M. J., Yilmaz, O. H., Iwashita, T., Yilmaz, O. H., Terhorst, C., and Morrison, S. J. (2005) SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* **121,** 1109–1121
34. Kiel, M. J., He, S., Ashkenazi, R., Gentry, S. N., Teta, M., Kushner, J. A., Jackson, T. L., and Morrison, S. J. (2007) Haematopoietic stem cells do not asymmetrically segregate chromosomes or retain BrdU. *Nature* **449,** 238–242
35. Nishinakamura, R., Nakayama, N., Hirabayashi, Y., Inoue, T., Aud, D., McNeil, T., Azuma, S., Yoshida, S., Toyoda, Y., Arai, K., *et al.* (1995) Mice deficient for the IL-3/GM-CSF/IL-5 βc receptor exhibit lung pathology and impaired immune response, while βIL3 receptor-deficient mice are normal. *Immunity* **2,** 211–222
36. Du, J., Liu, Y., Meline, B., Kong, G., Tan, L. X., Lo, J. C., Wang, J., Ranheim, E., Zhang, L., Chang, Y. I., Ryu, M. J., Zhang, J. F., and Zhang, J. (2013) Loss of CD44 attenuates aberrant GM-CSF signaling in Kras G12D hematopoietic progenitor/precursor cells and prolongs the survival of diseased animals. *Leukemia* **27,** 754–757
37. Matsuura, S., Yan, M., Lo, M. C., Ahn, E. Y., Weng, S., Dangoor, D., Matin, M., Higashi, T., Feng, G. S., and Zhang, D. E. (2012) Negative effects of GM-CSF signaling in a murine model of t(8;21)-induced leukemia. *Blood* **119,** 3155–3163