Deficiency of β Common Receptor Moderately Attenuates the Progression of Myeloproliferative Neoplasm in NrasG12D+/+ 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

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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 phenotypes in human and mouse. However, signaling plays a central role in establishing and maintaining (JMML/MP-CMML). Numerous studies suggest that GM-CSF myeloproliferative variant of chronic myelomonocytic leukemia (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 variant of CMML: MP, myeloid progenitor; HSC, hematopoietic stem cell; βc, common β chain of GM-CSF receptor; pl-pC, polyinosinic-polycytidylic acid; EdU, 5-ethynyl-2′-deoxyuridine; AML, acute myeloid leukemia; m, mouse; p, phospho.
**β Common Receptor in Nras**\(^{G12D/+}\) **-induced CMML**

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 (βc\(^{-/-}\)) were obtained from The Jackson Laboratory (stock number 005940). Nras\(^{LSL G12D/+}\); βc\(^{-/-}\) mice were crossed to Mx1-Cre; βc\(^{-/-}\) to generate compound mice Nras\(^{LSL G12D/+}\); Mx1-Cre; βc\(^{-/-}\). 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 (pl-pC; GE Healthcare) every other day for two doses. The day of first pl-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 FACS Calibur 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 (eBioID3), Thy1.2 (53-2.1), TER119 (TER-119), B220 (RA3-6B2), IgM (eBio121-15F9), IL-7Rα (B12-1), CD41 (eBio-MWReg30), CD48 (HM48-1), Sca1 (D7), cKit (2B8), and CD34 (RAM34). FcγRII/III (2.4G2) was purchased from BD Biosciences. CD150 (TC15-12F12) 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-
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<sup>+</sup>/H<sub>100</sub>2<sup>-</sup>/low cKit<sup>-</sup>/H<sub>100</sub>1<sup>-</sup> and Lin<sup>+</sup>/H<sub>100</sub>2<sup>-</sup>/low cKit<sup>-</sup>/H<sub>100</sub>2<sup>-</sup> 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 F(ab')<sub>2</sub> 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<sup>+</sup> cells were enriched using an AutoMACS (Miltenyi). Enriched cells were first stained with FITC-conjugated antibodies against Mac<sup>1</sup>/Gr<sup>1</sup>, CD41, CD48, B220, and TER119, and phycoerythrin-conjugated anti-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 Scientific).
Figure 2. βc deficiency abolishes GM-CSF signaling but preserves IL-3 signaling in Nras\textsuperscript{G12D/+} cells. A and B, total bone marrow cells isolated from control, Nras\textsuperscript{G12D/−}, or Nras\textsuperscript{G12D/−}; βc\textsuperscript{−/−} 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\textsuperscript{low}/cKit\textsuperscript{−} cells (Pro), whereas myeloid precursors are enriched in Lin\textsuperscript{low}/cKit\textsuperscript{+} cells (Pre). Data are presented as mean ± S.D. *, p < 0.05; **, p < 0.01; ***, p < 0.001.
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<sup>LSL.G12D/+</sup>; Mx1-Cre and Nras<sup>LSL.G12D/+</sup>; Mx1-Cre; βc<sup>-/-</sup> mice (Fig. 1, A and B). At 5–7 weeks of age, these mice were administrated with pl-pC, which stimulates endogenous interferon production and induces Cre expression in various tissues but predominantly in 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 pl-pC injection is defined as Day 1. After two rounds of pl-pC injection, all mice were sacrificed on Day 12. We refer to the pl-pC-treated compound mice as control mice (Fig. 1C). Flow cytometric analysis demonstrated that the percentages of granulocytes (Mac<sup>+</sup>) and monocytes (Mac1<sup>+</sup>) were grossly normal, with unremarkable white blood cell counts, hematocrit, and platelet counts (Fig. 1D) and normal myeloid differentiation in bone marrow and peripheral blood (Fig. 1E). However, Nras<sup>G12D/+</sup> mice showed moderately but significantly enlarged spleen when compared with control mice (Fig. 1C). Flow cytometric analysis demonstrated that the percentages of granulocytes (Mac<sup>+</sup>Gr1<sup>+</sup>) and monocytes (Mac1<sup>+</sup>Gr1<sup>-</sup>) were significantly increased in Nras<sup>G12D/+</sup> spleens when compared with control mice.

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

Deletion of βC does not affect Nras<sup>G12D/+</sup> HSCs. Mice were treated with pl-pC and euthanized on Day 12 for analysis as described under “Materials and Methods.” A–C, Lin<sup>+</sup>CD41<sup>-</sup>CD48<sup>-</sup> ckit<sup>+</sup> Sca1<sup>+</sup> CD150<sup>-</sup> HSCs (A), Lin<sup>+</sup>CD41<sup>-</sup>CD48<sup>-</sup> ckit<sup>+</sup> Sca1<sup>+</sup> CD150<sup>-</sup> 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<sup>5</sup> bone marrow cells from control, Nras<sup>G12D/+</sup>, or Nras<sup>G12D/+; βc<sup>-/-</sup></sup> mice were transplanted with the same number of competitor cells into lethally irradiated mice. The percentages of donor-derived cells (CD45.2<sup>+</sup>) 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.
β Common Receptor in NrasG12D/+ -induced CMML

A

B

FIGURE 4. Loss of βc greatly reduces NrasG12D/+ -induced spontaneous colony formation. Mice were treated with pl-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 × 10^6 bone marrow cells isolated from control, NrasG12D/+, or NrasG12D/; βc−/− 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. 1E). Notably, the splenomegaly and percentage of splenic monocytes were significantly reduced in NrasG12D/+; βc−/− mice when compared with those in NrasG12D/+ 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 NrasG12D/+; βc−/− mice.

Loss of βc Abolishes GM-CSF Signaling but Preserves IL-3 Signaling in NrasG12D/+ Cells—To determine whether loss of βc affects cytokine signaling in NrasG12D/+ cells, we studied GM-CSF signaling and IL-3 signaling in Linlow−/− cKit+ cells (enriched for MPs, early progenitor cells that give rise to various types of myeloid cells) and Linlow−/− cKit+ cells (enriched for myeloid precursors, immature myeloid cells that are downstream of MPs) from control, NrasG12D/+, and NrasG12D/+; βc−/− mice (Fig. 2). We found that GM-CSF- and IL-3-evoked ERK1/2 and STAT5 activation in NrasG12D/+ MPs was largely comparable with that in control cells, whereas NrasG12D/+ 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 NrasG12D/+; βc−/− cells (Fig. 2A), whereas IL-3 signaling remained intact (Fig. 2B).

βc Is Dispensable for NrasG12D/+ HSCs—Because NrasG12D/+ 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, NrasG12D/+, and NrasG12D/+; βc−/− 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 NrasG12D/+; βc−/− mice were moderately but significantly increased when compared with those in control mice but comparable with those in NrasG12D/+ mice (Fig. 3A). Concomitantly, the compartments of multipotent progenitors (Lin− CD41− CD48− cKit+ Sca1+ CD150+) (33) and LSK cells in bone marrow and/or spleen of NrasG12D/+; βc−/− mice were also expanded but indistinguishable from those in NrasG12D/+ 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 NrasG12D/+ HSCs (27), we performed EdU incorporation analysis. The percentages of cycling NrasG12D/+; βc+/− and NrasG12D/+; βc−/− HSCs seemed to be higher than control HSCs but comparable with NrasG12D/+ HSCs (Fig. 3D). To investigate further whether deletion of βc affects increased self-renewal of NrasG12D/+ HSCs (27), we transplanted 2.5 × 10^6 total bone marrow cells (CD45.2+) isolated from control, NrasG12D/+, and NrasG12D/+; βc−/− 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 NrasG12D/+; βc−/− 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 NrasG12D/+ cells (Fig. 3E). These data
Figure 5. Deletion of βc slows down the progression of NrasG12D+/−-induced hematopoietic malignancies. Lethally irradiated mice were transplanted with 2.5 × 10⁶ total bone marrow cells from control, NrasG12D+/−, or NrasG12D+/−; 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 NrasG12D+/− or NrasG12D+/−; βc−/− cells. T-ALL, T-cell acute lymphoblastic leukemia/lymphoma. C, spleen and liver weight of moribund CMML-NrasG12D+/− or CMML-NrasG12D+/−; βc−/− mice. D, complete blood count was performed on peripheral blood samples collected from moribund CMML-NrasG12D+/− or CMML-NrasG12D+/−; 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-NrasG12D+/− and CMML-NrasG12D+/−; βc−/− mice using myeloid lineage-specific markers. F, representative spleen histologic H&E sections from moribund CMML-NrasG12D+/− and CMML-NrasG12D+/−; βc−/− mice and age-matched control mice. Data are presented as mean ± S.D. * p < 0.05; ** p < 0.01.
suggest that deletion of βc does not affect increased self-renewal of Nras\textsuperscript{G12D/+} HSCs. Together, our results demonstrate that βc is dispensable for Nras\textsuperscript{G12D/+} HSCs.

Loss of βc Abolishes Oncogenic Nras-induced Spontaneous Colony Formation—To test whether loss of βc affects MP expansion in Nras\textsuperscript{G12D/+} mice, we analyzed the MP (Lin\textsuperscript-{IL7Ra} Sca1\textsuperscript{-} cKit\textsuperscript{+}) compartment in control, Nras\textsuperscript{G12D/+}, and Nras\textsuperscript{G12D/+}; βc\textsuperscript{-/-} mice. The absolute numbers of MPs, including common myeloid progenitors, granulocyte-monocyte progenitors, and megakaryocyte-erythroid progenitors, in Nras\textsuperscript{G12D/+} and Nras\textsuperscript{G12D/+}; βc\textsuperscript{-/-} bone marrow were comparable with each other, and both numbers were significantly higher than those in control bone marrow (Fig. 4A). A similar trend was also observed in spleen (Fig. 4A). 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\textsuperscript{G12D/+} mice form a significant number of colonies in the absence of cytokines (10, 11). To determine whether the spontaneous colony formation of Nras\textsuperscript{G12D/+} cells depends on βc-mediated signaling, we isolated bone marrow cells from control, Nras\textsuperscript{G12D/+}, and Nras\textsuperscript{G12D/+}; βc\textsuperscript{-/-} mice and plated them in semi-solid medium in the absence of cytokines. Consistent with previous studies (10, 11), Nras\textsuperscript{G12D/+} cells formed a significant number of colonies, whereas Nras\textsuperscript{G12D/+}; βc\textsuperscript{-/-} cells formed a much lower number of colonies (Fig. 4B). In the presence of mGM-CSF or mIL-3, Nras\textsuperscript{G12D/+} cells formed significantly more and bigger colonies than control cells (Fig. 4B). As expected, Nras\textsuperscript{G12D/+}; βc\textsuperscript{-/-} 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\textsuperscript{G12D/+} cells in the presence of mIL-3, consistent with our signaling studies (Fig. 4B). Our results suggest that the spontaneous colony formation of Nras\textsuperscript{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\textsuperscript{G12D/+}, and Nras\textsuperscript{G12D/+}; βc\textsuperscript{-/-} mice together with competitor cells into lethally irradiated mice (Fig. 5). Consistent with our previous study (10), ~97% of recipients transplanted with Nras\textsuperscript{G12D/+} cells developed a CMML-like disease and ~7% developed acute T-cell lymphoblastic leukemia/lymphoma. Similarly, ~94% of recipients with Nras\textsuperscript{G12D/+}; βc\textsuperscript{-/-} cells developed a CMML-like disease and ~12% developed T-cell lymphoblastic leukemia/lymphoma (Fig. 5B). Some mice developed both diseases. However, recipients transplanted with Nras\textsuperscript{G12D/+}; βc\textsuperscript{-/-} cells survived significantly longer than
those with Nras\textsuperscript{G12D/+} cells (median survival: 537 days versus 357 days) (Fig. 5A). Despite different survival curves, both groups of mice with CMML displayed similar disease phenotypes at the moribund stage, including markedly enlarged spleen (Fig. 5C) with significant extramedullary hematopoiesis (Fig. 5F), increased white blood cell counts and anemia (Fig. 5D), and a predominant expansion of granulocytes and/or monocytes in hematopoietic tissues (Fig. 5E). These results indicate that deletion of βc cannot abrogate oncogenic Nras-induced CMML formation but it does significantly delay CMML progression.

Deletion of βc in Nras\textsuperscript{G12D/+} Mice Promotes Hepatic Histiocytic Sarcomas with Atypical Morphology—Our previous results show that ~50% of primary Nras\textsuperscript{G12D/+} mice (7 out of 15) died with hepatic histiocytic sarcoma within a year after pl-pC injections (10). Although the median survival of Nras\textsuperscript{G12D/+; βc\textsuperscript{-/-}} mice was indistinguishable from that of Nras\textsuperscript{G12D/+} mice, the disease latency was prolonged (Fig. 6). Like Nras\textsuperscript{G12D/+} mice, most of the Nras\textsuperscript{G12D/+; βc\textsuperscript{-/-}} mice (6 out of 8) developed CMML-like phenotypes with increased monocytosis in peripheral blood (Fig. 6C). Two out of three Nras\textsuperscript{G12D/+; βc\textsuperscript{-/-}} 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. 7A), we initially considered whether this might represent a non-hematopoietic tumor type, but negative pan-keratin staining (Fig. 7B) 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\textsuperscript{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\textsuperscript{G12D/+} cells but IL-3 signaling is preserved (Fig. 2). Consequently, loss of βc does not affect Nras\textsuperscript{G12D/+} HSC functions (Fig. 3) and therefore does not abrogate CMML in Nras\textsuperscript{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\textsuperscript{G12D/+} cells (Fig. 5). Our findings are summarized in Fig. 8. We previously reported that in the Nras\textsuperscript{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\textsuperscript{G12D/+} HSCs as well; loss of βc does not affect the expansion, increased proliferation and self-renewal, and myeloid differentiation bias in Nras\textsuperscript{G12D/+} HSCs (Figs. 3 and 4). We believe that βc is also dispensable for Nf1\textsuperscript{-/-} HSCs. Therefore, it is not surprising that the MP compartment remains expanded in

\begin{figure}
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\caption{Analysis of livers from moribund Nras\textsuperscript{G12D/+} and Nras\textsuperscript{G12D/+; βc\textsuperscript{-/-}} 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.}
\end{figure}

\begin{figure}
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\includegraphics[width=\textwidth]{fig8}
\caption{Schematic picture illustrating the role of βc-mediated signaling in Nras\textsuperscript{G12D/+}-induced CMML. UPD, uniparental disomy.}
\end{figure}
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\(\text{Nras}^{G12D/+}; \beta c^{-/-}\) (Fig. 4) and \(\text{Nf1}^{-/-}; \beta c^{-/-}\) mice and that deletion of \(\beta c\) 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 \(\beta c\)-mediated GM-CSF signaling, \(\beta c\) deficiency indeed significantly reduces \(\text{Nras}^{G12D/+}\)-induced splenomegaly (Fig. 1C) and spontaneous colony formation (Fig. 4B) and prolongs the survival of CMML mice (Fig. 5A), 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 JMM patients. Although we and others did not see AML genesis in \(\text{Nras}^{G12D/+}; \beta c^{-/-}\) and \(\text{Nf1}^{-/-}; \beta c^{-/-}\) mice, we could not rule out the possibility that long-term inhibition of GM-CSF signaling in JMM/CML 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.

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