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Letter to the Editor

# Senescent bone marrow microenvironment promotes *Nras*-mutant leukemia

**Dear Editor,**

In recent years, there is growing interest regarding the roles of senescent bone marrow (BM) microenvironment in the initiation of leukemia. Aged mice transplanted with AML1-ETO (AML1-ETO fusion protein)-positive hematopoietic stem cells (HSCs) present with a significant increase in the frequency of AML-ETO-positive early progenitor cells in BM as well as an increase of immature myeloid cells compared to young recipients (Vas et al., 2012). BM mesenchymal stem cells (MSCs) from myelodysplastic syndromes (MDS) animal models and MDS patients exhibit impaired proliferation and differentiation potentials, abnormal cytokine secretion, and dysregulated gene expression profile (Lopez-Villar et al., 2009; Geyh et al., 2013; Mattiucci et al., 2018). However, the causal relationship between senescent BM microenvironment and leukemia development is unclear. Whether senescent BM microenvironment initiates or accelerates leukemia development remains unknown.

To address above questions, we investigated the roles of BM MSCs in the development and progression of myelodysplastic/myeloproliferative neoplasms (MDS/MPN), using an oncogenic *Nras*-mutant mouse model. NRAS-mutant chronic myelomonocytic leukemia (CMML) belongs to MDS/MPN, which is more frequent in elderly people than in younger. We previously reported that the mouse model carrying an endogenous mutant *NrasG12D* allele developed CMML-like leukemia, which belongs to a subtype of MDS/MPN (Wang et al., 2010, 2011, 2013). The increasing kinetics of $CD11b^+$ cells in peripheral blood (PB) projected the disease development. Here, we found that BM MSCs acquired cellular aging-like alterations in NrasG12D mutation-induced leukemic mice, including aging-like morphology (Figure 1A and B) and reduction of endosteal osteoblasts (Figure 1C and D; Supplementary Figure S1). Additionally, ~80% of leukemic MSCs were SA-β-Gal-positive (Figure 1E and F), which confirmed the senescent phenotype of leukemic MSCs. To test whether the senescent BM microenvironment contributes to acceleration of leukemia progression, we transplanted the BM leukemic cells ($CD45.2^+$) into sublethally irradiated young (2 months old, $CD45.1^+$) or old (18 months old, $CD45.1^+$) recipients (Figure 1G). We used the old recipients transplanted with wild-type (WT, $CD45.1^+$) BM cells as transplantation control. The percentages of donor chimerism ($CD45.2^+$) in the PB of old and young recipients were both >95% at Week 16 (Figure 1H). The tumor burden ($CD45.2^+CD11b^+$% in PB) was subsequently monitored every month post-transplantation to assess the leukemia progression. Tumor burden began to increase faster in old recipients than young recipients after 20 weeks of post-transplantation (Figure 1I). Both old-recipient and young-recipient leukemic mice showed significant splenomegaly and leukocytosis (Figure 1J). In NrasG12D mutation-induced leukemia model, we arbitrarily set ≥60% as high tumor burden and <60% as no/low tumor burden. With this definition of disease, the median duration of no/low tumor burden stage for old recipients was 184 days, compared with 324 days for young recipients ($P < 0.001$; Figure 1K). These results indicated that senescent BM microenvironment accelerates leukemia progression.

Subsequently, we attempted to rejuvenate the senescent BM microenvironment of leukemic mice by intra-BM transfusion of young or old MSCs. In a previous report, we have proved that intra-BM transfusion of young MSCs can suppress leukemia via functional restoration of BM microenvironment. We adopted the same injection procedure as previously reported to transfuse young or old MSCs (Supplementary Figure S2) into leukemic mice (tumor burden ~60%). The tumor burden continued to increase (over 60%) in leukemic mice treated with old MSCs, while the tumor burden continuously decreased to ~40% after 16 weeks of post-injection in leukemic mice treated with young MSCs (Figure 1L). This was related to the recovery of normal hematopoiesis, demonstrated by reduction of HSC frequency in BM, and rebalanced lineage cells in PB (Supplementary Figure S3). Consequently, survival of old-MSC-treated leukemic mice was significantly shorter than that of young-MSC-treated leukemic mice (old MSCs: 55.5 days, young MSCs: 120 days, $P < 0.001$; Figure 1M). Additionally, we did not observe recovery of endosteal osteoblasts in old-MSC-treated leukemic mice, while endosteal osteoblasts re-occurred along the lining of young-MSC-treated

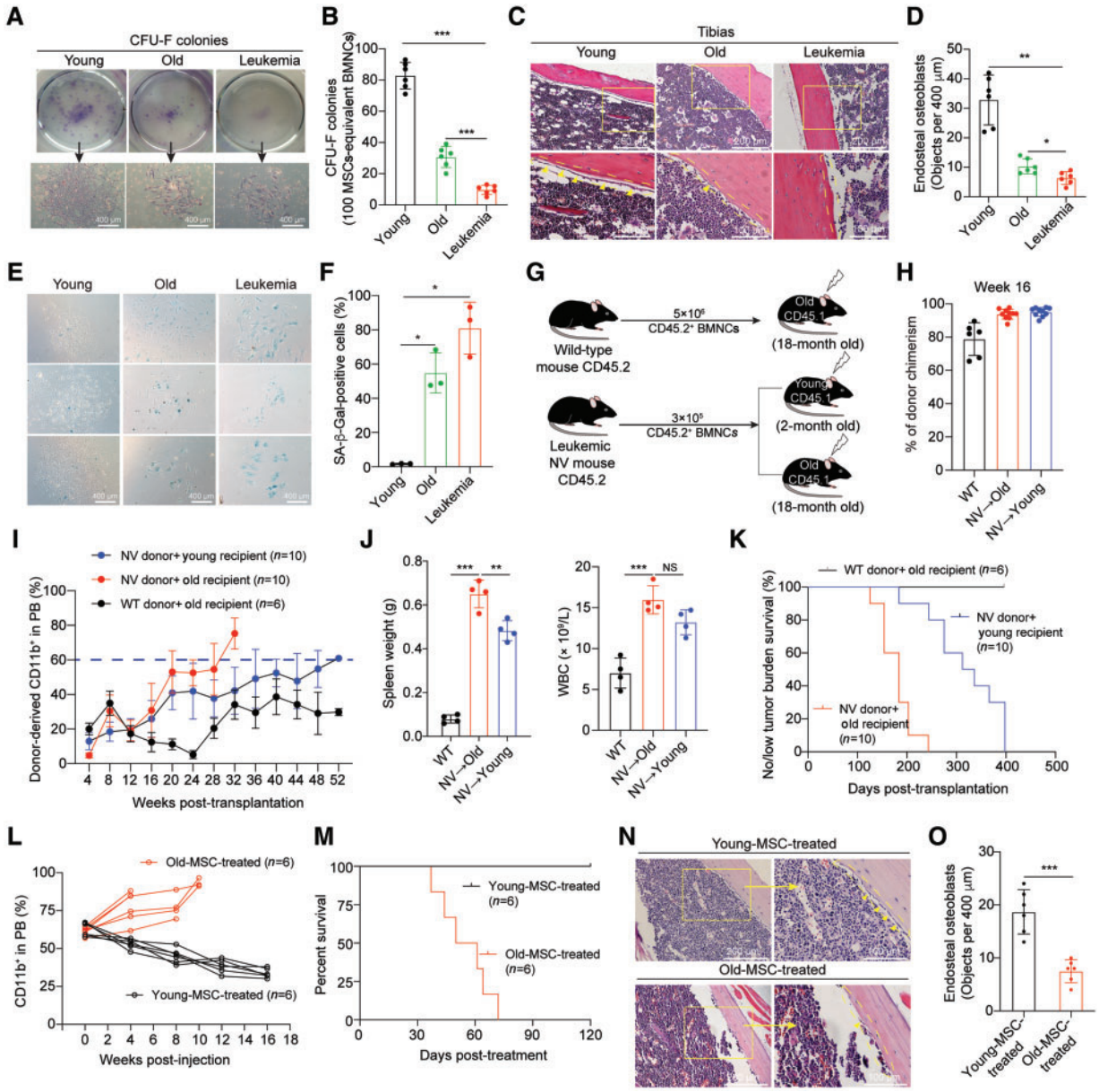


**Figure 1** Senescent BM microenvironment promotes *Nras*-mutant leukemia. (**A** and **B**) Representative photomicrographs (**A**) and statistical analysis (**B**) of colony-forming units (CFU-F) colonies of young MSCs, old MSCs, and leukemia MSCs by Giemsa staining. Scale bar, 400 μm. $n = 6$ mice for each group. BMNCs, bone marrow nucleated cells. (**C** and **D**) Hematoxylin and eosin staining (**C**) and quantity (**D**) of osteoblasts in the endosteal lining of tibias/femurs of young mice (2 months old), old mice (18 months old), and leukemic mice. $n = 6$ mice for each group. (**E** and **F**) Representative images (**E**) and quantitative analysis (**F**) of SA-β-Gal staining in MSCs at Day 10 from young, old, and leukemia mice. The SA-β-Gal-positive cells exhibited blue color. Scale bar, 400 μm. $n = 3$ mice. (**G**) Schematic experimental setup. $CD45.2^+$ BMNCs from LSL Nras/+; Vav-Cre mice (NV mice) were sorted and transplanted into sublethally irradiated (6.5 Gy) individual young (2 months) or old (18 months) recipients (CD45.1 strain) with a cell dose of 0.3 million per recipient. (**H**) Percentage of donor-derived cells ($CD45.2^+$) detected in PB of recipients at Week 16. (**I**) Dynamic percentage of tumor burden ($CD45.2^+CD11b^+$ cells, donor-derived) in the PB of recipient mice at the indicated time points post-transplantation ($n = 6$–10 mice for each group, two independent experiments). (**J**) Statistical analysis of spleen weight and white blood cell (WBC) count of WT recipients and diseased mice. (**K**) Kaplan–Meier no/low tumor burden survival curves of WT donor + old recipient (black line, $n = 6$, median survival = 396 days), NV donor + young recipient (blue line, $n = 10$, median survival = 324 days), and NV donor + old recipient (red line, $n = 10$, median survival = 184 days) are shown. Log-rank (Mantel–Cox) test, $P < 0.001$. (**L**) Kinetic analysis of tumor burden ($CD45.2^+CD11b^+$) of leukemic mice treated with young MSCs (black line, $n = 6$) or old MSCs (red line, $n = 6$). (**M**) Kaplan–Meier survival curves of young-MSC-treated (black line, $n = 6$, median survival = 120 days) and old-MSC-treated (red line, $n = 6$, median survival = 55.5 days) leukemic mice are shown. Log-rank (Mantel–Cox) test, $P < 0.001$. MSC treatment was terminated after 16 weeks. (**N** and **O**) Hematoxylin and eosin staining (**N**) and quantity (**O**) of recovered osteoblasts in endosteal lining of tibias/femurs of leukemic mice treated with young or old MSCs. $n = 6$ mice for each group. Data are represented as mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. NS, not significant.

leukemic BM (Figure 1N and O). It is possible that the insufficient therapeutic effects of donor old MSCs are due to their senescent characteristics. Tracing the dynamic and cell fate of donor MSCs *in vivo* would further help to reveal the potential mechanisms of therapeutic effects of young MSCs. In summary, these data demonstrated that intra-BM transfusion of young MSCs can rejuvenate the senescent BM microenvironment under *Nras*-mutant leukemia condition. Based on our recent work (Xia et al., 2020), although senescent BM microenvironment might occur in an MLL-AF9 acute myeloid leukemia mouse model, intra-BM transfusion of young MSCs was insufficient to suppress disease development. We will verify our hypothesis using more leukemic models in the future and explore the potential therapeutic effects of intra-BM transfusion of MSCs in other types of leukemia.

Taken together, our study highlights the roles of senescent BM microenvironment in the development and progression of leukemia. It is promising to take the rejuvenation of senescent BM microenvironment as an adjuvant therapeutic strategy to suppress leukemia development in late-stage MDS/MPN patients.

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