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RESEARCH HIGHLIGHT

Tif1γ controls the TGF-β receptor on hematopoietic stem cells: implication in physiological aging

Ronan Quéré[1,2], Jean-Noël Bastie[1,2,3], Laurent Delva[1,2]

[1]*UMR 866 Inserm, Dijon 21000, France*
[2]*Labex LipSTIC, Université de Bourgogne, Dijon 21000, France*
[3]*Hôpital Universitaire, Service d'Hématologie Clinique, Dijon 21000, France*

Correspondence: Ronan Quéré
E-mail: ronan.quere@inserm.fr
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Hematopoietic stem cell (HSC) aging has been directly linked to the development of several hematological disorders including myeloproliferative diseases. We recently described that in elderly mice (20-month-old), physiological aging of the hematopoietic system is associated with a decreased expression of the Transcription Intermediary Factor 1γ (Tif1γ) gene in HSCs. In young mice (4-month-old), deleted for the Tif1γ gene in HSCs (Tif1γ$^{-/-}$), the hematopoiesis aging phenotype is intensified. We discovered that Tif1γ controls the TGF-β receptor 1 (Tgfbr1) and finely regulates the number of myeloid-restricted HSCs in bone marrow. Altogether, we established that young Tif1γ$^{-/-}$ mice develop a phenotype of premature hematopoietic aging, which may explain their tendency to myeloproliferative disease. We identified two populations of HSCs specifically discriminated by Tgfbr1 expression and afforded evidence of the capture of myeloid-restricted (Tgfbr1^{hi}) and myeloid-lymphoid-balanced (Tgfbr1^{lo}) HSC. In conclusion, our study proves that Tif1γ can regulate the balance between lymphoid and myeloid HSCs, through a modulation of the TGF-β signaling.

Keywords: TGF-β receptor 1; hematopoietic stem cell; aging; Tif1γ; myeloid-biased HSC

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Alterations in the biological functions of old HSCs have been associated with the development of several hematological disorders, with the increased incidence of myeloproliferative diseases, leukemia and other hematological neoplasms [1]. It has become obvious that HSCs are heterogeneous given their differentiation capacities. Indeed, some HSCs harbor a low ability to differentiate into lymphoid cells and are considered as myeloid-biased whereas other HSCs exhibit the opposite properties. Adult HSCs produce all blood lineages during lifetime, but their capacity to generate lymphoid cells decreases with age, while myelopoiesis is enhanced [2, 3, 4]. Myeloid- and lymphoid-biased HSC populations seem to react differently to TGF-β signaling [5]. Therefore, the mechanisms by which age-related transformations in HSC are affected by TGF-β need to be described.

Mice deleted for the Tif1γ gene develop a myeloproliferative disease involving the proliferation of immature precursors of myelocytes and monocytes [6, 7, 8].

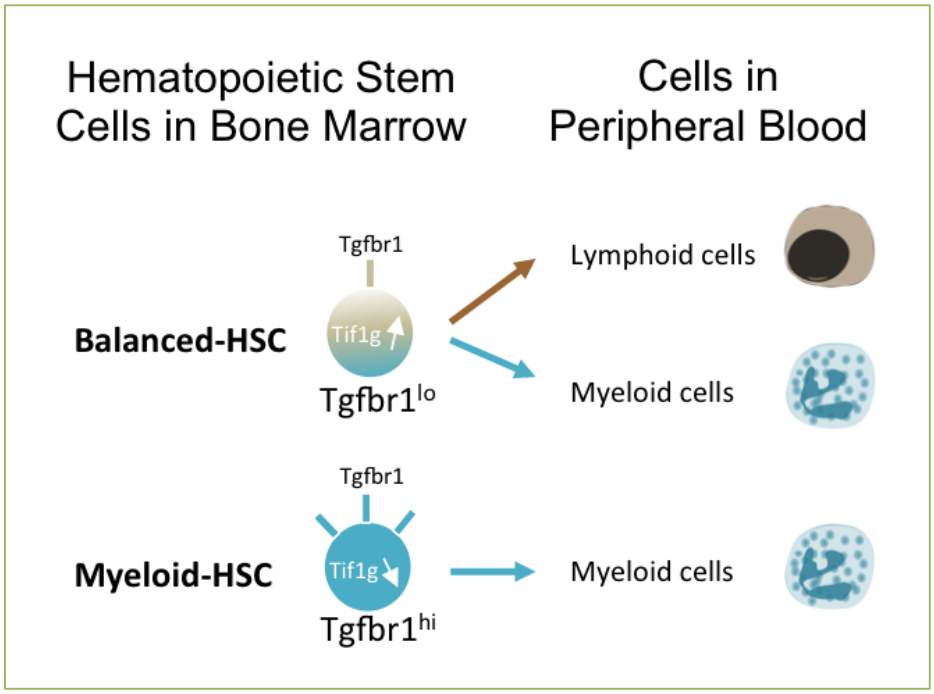

Figure 1. Tif1γ regulates the Tgfbr1 receptor level on myeloid-biased HSCs. We demonstrated that two populations of HSCs coexist, depending on the level of expression of the TGF-β1 receptor. While Tgfbr1^{lo} cells reconstituted both myeloid and lymphoid lineages, Tgfbr1^{hi} cells exhibited myeloid-biased differentiation, which is characterized by the exclusive production of myeloid cells in peripheral blood and very low reconstitution of both B and T lymphoid lineages. Furthermore, although Tgfbr1^{hi} cells generated more progenitors in bone marrow, those progenitors have reduced contribution to the total circulating white blood cells.

Therefore, the disease recapitulates important characters of the human chronic myelomonocytic leukemia (CMML) [6]. In humans, the mean age at the onset of this disease is over 70 years old, and is therefore possibly associated with the aging-related decreased fitness of HSCs. As there are important analogies between the physiological aging of hematopoiesis and the HSC premature aging phenotype observed in young Tif1γ$^{-/-}$ mice, we recently used this newly established model to investigate the impact of Tif1γ on hematopoietic aging [9].

Tif1γ$^{-/-}$ HSCs displayed an increased tendency to differentiate towards myeloid rather than lymphoid lineages, which contributes to the regression of lymphopoiesis that accompanies aging. Despite their proliferation in bone marrow, Tif1γ$^{-/-}$ HSCs showed decreased capacity to differentiate and generate white cells in peripheral blood, which also corroborates the aging phenotype of HSCs. Furthermore, it turned out in our study that young Tif1γ$^{-/-}$ HSC supported the stem cell-autonomous parameters of HSC aging. When Tif1γ$^{-/-}$ HSCs were transplanted into recipient mice, they exhibited similar properties of deficient competitive fitness, reduced ability to produce lymphoid and erythroid progenitors, and increased capacity to generate more myeloid progenitors. The accumulation of DNA repair defects characterizes aging of HSC [10, 11] and we also found an increased number of DNA foci, which support the genomic instability in Tif1γ$^{-/-}$ HSC. We furthermore observed an altered endosteal location of Tif1γ$^{-/-}$ HSC, which reinforces the aged phenotype. Finally, Tif1γ$^{-/-}$ primitive hematopoietic cells showed alterations in their gene expression programs that are much closed to physiological aging. Our study therefore reported that 4-month-old mice deleted for Tif1γ in HSC developed a phenotype of premature hematopoietic aging that may explain their predisposition to myeloproliferative disease.

Since Tif1γ has been shown to affect cell response to the TGF-β superfamily in different tissues [12, 13, 14, 15], we investigated the expression of several key proteins involved in the TGF-β signaling. Surprisingly, the expression of the TGF-β receptor 1 (Tgfbr1, Alk5) was found more than 4 times greater in the Tif1γ$^{-/-}$ HSCs compared with the control cells. Furthermore, treatment with recombinant TGF-β1 had a considerable antiproliferative effect on Tif1γ$^{-/-}$ cells, compared to the control cells, supporting the hypothesis that loss of Tif1γ would make HSC more sensitive to TGF-β1. Besides its role as a transcription co-factor, Tif1γ is known to display polyubiquitin ligase activity [13, 15]. In our study, we discovered that Tif1γ indeed regulates the TGF-β1

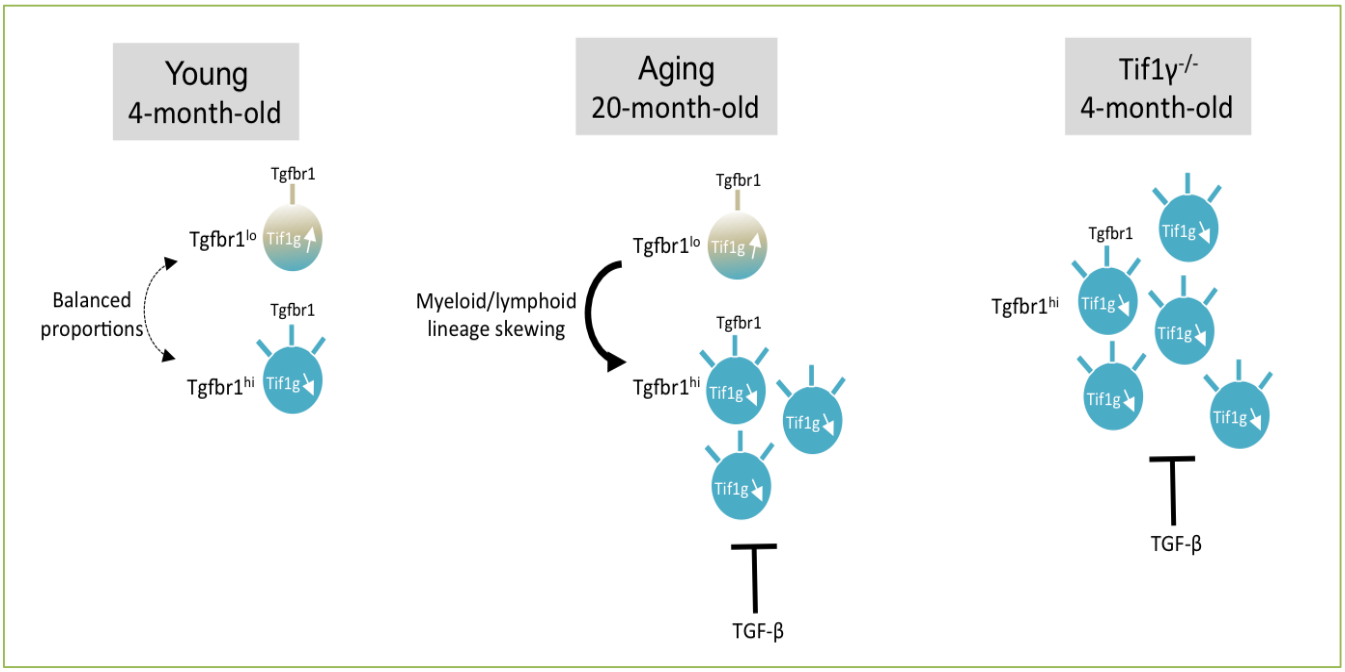

Figure 2. Tif1γ$^{-/-}$ and old HSCs expressed high level of the Tgfbr1 receptor and are more sensitive to TGF-β signaling. We found that myeloid-biased Tgfbr1^{hi} cells become the dominant HSC subtype in old mice during physiological hematopoietic aging and in young Tif1γ$^{-/-}$ mice, which developed a premature hematopoietic aging phenotype. Under administration of recombinant TGF-β1 *in vivo*, Tif1γ$^{-/-}$ and old myeloid HSCs are more sensitive, stop their proliferation and enter in quiescent state.

receptor turnover via its ubiquitin ligase activity.

To provide evidence of heterogeneity and to discriminate HSCs according to Tgfbr1 expression, we isolated two subpopulations of cells expressing Tgfbr1 with either high (Tgfbr1^{hi}) or low (Tgfbr1^{lo}) rates (Fig. 1). Each population was transplanted in a competitive manner into lethally irradiated recipient mice. Sixteen weeks after bone marrow reconstitution, we discovered that Tgfbr1^{hi} cells showed significantly lower contribution to the total circulating white blood cells. Furthermore, while Tgfbr1^{lo} cells reconstituted both myeloid and lymphoid lineages, Tgfbr1^{hi} cells exhibited myeloid-restricted differentiation, which is characterized by the exclusive production of myeloid cells in peripheral blood and very low lymphoid lineage reconstitution (Fig. 1). In conclusion, our study identified Tgfbr1 as a novel positive marker to differentiate myeloid-derived HSC.

The TGF-β signaling pathway has long been connected with HSC quiescence [16, 17, 18]. Since Tif1γ$^{-/-}$ and old HSCs expressed high levels of Tgfbr1 (Fig. 2), we analyzed the effect of high dose of recombinant TGF-β1 on HSCs in bone marrow and observed an increased proportion of dormant HSCs from Tif1γ$^{-/-}$ and old mice, whereas the treatment did not affect the quiescence of young HSCs. Thus, Tif1γ$^{-/-}$ and old Tgfbr1^{hi} HSCs were more sensitive to TGF-β signaling under high dose of TGF-β1, that caused ant proliferative effect and greater dormancy in bone marrow, which explains the reduced number of myeloid cells in peripheral blood. In our study, we therefore discovered that administration of high dose of recombinant TGF-β1 could rescue the biased myeloproliferation in old and Tif1γ$^{-/-}$ mice. Importantly, this effect was reversed upon addition of the TGF-β type I receptor inhibitor SB431542 which is known to remove HSCs from their quiescence, leading to an increased myeloproliferation in Tif1γ$^{-/-}$ and old mice.

Conflicting interests

The authors have declared that no competing interests exist.

Acknowledgments

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