



# Promyelocytic leukemia protein in mesenchymal stem cells is essential for leukemia progression

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## Abstract

The dynamic interactions between leukemic cells and cells resident within the bone marrow microenvironment are vital for leukemia progression. The lack of detailed knowledge about the cellular and molecular mechanisms involved in this cross-talk restricts the design of effective treatments. Guarnerio et al. (2018) by using state-of-the-art techniques, including sophisticated Cre/loxP technologies in combination with leukemia mouse models, reveal that mesenchymal stem cells via promyelocytic leukemia protein (Pml) maintain leukemic cells in the bone marrow niche. Strikingly, genetic deletion of Pml in mesenchymal stem cells raised survival of leukemic mice under chemotherapeutic treatment. The emerging knowledge from this research provides a novel target in the bone marrow niche for therapeutic benefit in leukemia.

**Keywords** Pml · Leukemia · Mesenchymal stem cells · Niche

## Introduction

Cancer is the leading cause of death worldwide [1]. The hematologic cancers, leukemias, are caused by the acquisition of malignant mutations in hematopoietic progenitor cells which confer their clonal expansion, aberrant self-renewal capacity, allowing them to proliferate indefinitely without differentiation, and prejudicing their normal functions [2]. Leukemia is characterized by the unrestrained formation of leukemic cells and defective normal hematopoiesis [3]. Despite significant improvements in the outcome of leukemic patients due to modern chemotherapeutic treatments combined with bone

marrow transplants, leukemia relapse after therapy is still common.

Leukemia, similarly to other malignancies, depends not only on intrinsic genetic alterations but is supported by cooperation with bystander cells residing in the bone marrow microenvironment [4, 5]. Signals emanating from these cells are essential for leukemic clones' maintenance, supporting survival, resistance to chemotherapy, proliferation, and migration [6]. Albeit the importance of the microenvironment for leukemic progression is well accepted, the detailed mechanisms involved in the complex interplay between the cells present in the leukemic niche and cancer cells are poorly understood. Deep understanding of the active involvement niche cells in shaping the leukemic microenvironment may offer novel clues for designing therapeutic strategies. The discovery of molecules that strengthen the niche-mediated resistance to chemotherapy challenges the eradication of residual leukemic cells hiding in the bone marrow microenvironment. Elucidating the effect of microenvironmental cues on leukemic cells will bring novel targets to achieve complete remission.

Bone marrow niches within the bones provide a sanctuary for leukemic cells to evade death. Even though it is well accepted that bone marrow stromal cells promote leukemic cells resistance to chemotherapy [7], the details of the cellular and molecular mechanisms involved in this support remain

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unclear. Mesenchymal stem cells are found throughout the body [8, 9]. These cells are able to self-renew and differentiate into various cell populations. In the bone marrow, they provide a niche for growth, differentiation, and survival of normal and malignant hematopoietic cells [10–12]. Now, in a recent article in *Nature Communications*, Guarnerio and colleagues reveal that the promyelocytic leukemia protein (Pml) in mesenchymal stem cells is as a therapeutic target for leukemia [13]. The authors investigated the role of mesenchymal stem cells in leukemia progression by using state-of-the-art techniques, including in vivo serial transplantation, sophisticated Cre/loxP technologies in combination with leukemia mouse models, and in vitro elegant co-culture assays. Using Prx1-Cre/Pml-floxed mice, these experiments revealed that mesenchymal stem cells expand after Pml loss in the bone marrow [13]. Interestingly, Guarnerio and colleagues showed that leukemic cell growth decreased in the bone marrow of Pml knockout mice as compared with controls, suggesting that sustainability of leukemic cells within the bone marrow depends on the expression of Pml in niche cells. Strikingly, deletion of Pml in mesenchymal stem cells increased survival of mice transplanted with leukemic cells under chemotherapeutic treatment via induction of leukemic cell cycle arrest and inhibiting leukemic reconstitution ability [13]. Moreover, Guarnerio and colleagues discovered that several pro-inflammatory molecules are downregulated post-deletion of Pml in cultured mesenchymal stem cells. Using blocking antibodies and recombinant proteins in co-cultures of leukemic cells with mesenchymal stem cells, the authors showed that chemokine (C-X-C motif) ligand 1 (CXCL1) and interleukin 6 (IL6) production depends on mesenchymal stem cells' Pml, and this Pml is important maintenance of leukemic cells by mesenchymal stem cells. Thus, this study brings novel targets for leukemia, which is urgently needed in the clinic (Fig. 1).

Here, we discuss the findings from this study and evaluate recent advances in our understanding of the leukemic micro-environment in the bone marrow.

## Perspectives/future directions

### Specificity of the use of Prx-Cre drivers

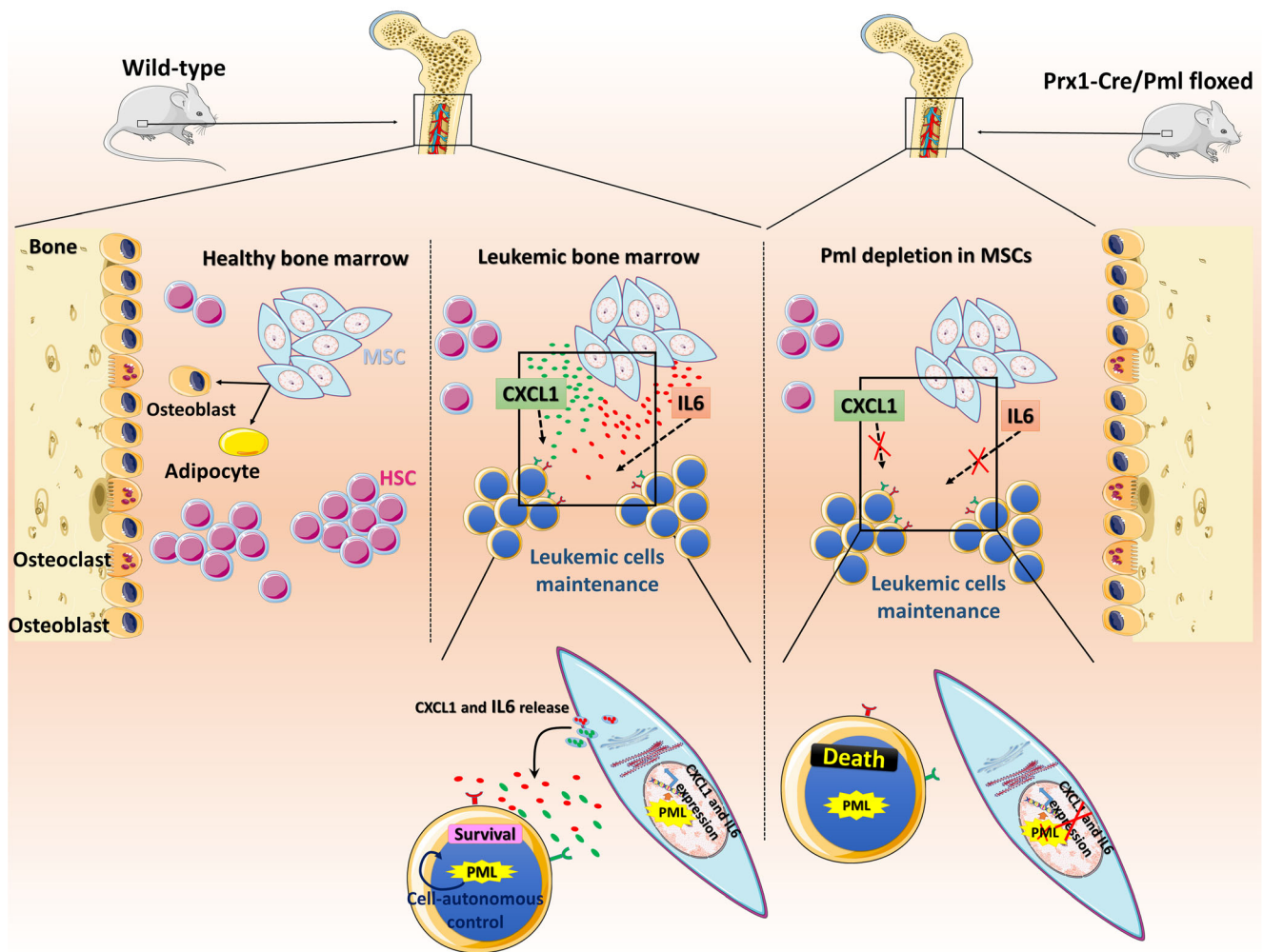
The use of conventional knockout mouse models has been proven useful to understand the role of key genes in physiological and pathological states. Nevertheless, these technologies produce broad changes in gene function throughout the body since embryonic stages, affecting several cell populations. Thus, they are limited in that they do little to contribute with knowledge about specific roles of a gene in a precise cell type. Since the molecular functions of proteins may depend on the particular cell subset in which they are expressed, restricting gene manipulation to unique cells in the bone marrow may be useful to

comprehend protein functions. Thus, conditional gene manipulation methods offer mighty alternatives [14–18]. The main findings from this study are based on the data obtained from Prx1-Cre/Pml-floxed mice [13]. It is known that paired-related homeobox 1 (Prx1) gene is expressed in mesenchymal stem cells [19]. Note, however, that expression of Prx1 is not restricted to mesenchymal stem cells. For instance, cells from the adipogenic lineage also may express Prx1 [20]. Additionally, Prx1 is broadly expressed early during limb bud mesoderm development [21, 22]. Consistent with this, Prx1-Cre mice may exhibit Cre recombinase activity in various cell populations during embryogenesis, in addition to mesenchymal stem cells [20]. Therefore, it is possible that the effect on leukemic cells could be due to a cell distinct from mesenchymal stem cells, in which Pml was also deleted in Prx1-Cre/Pml-floxed mice. Because of this, to avoid Cre recombinase activity in other cell types during development, a mouse model in which recombination only occur in adult Prx1-expressing cells (mesenchymal stem cells) should be used in future studies, i.e., Prx1-CreER mice [23]. In Prx1-CreER/Pml-floxed mice, it will be possible to temporally control Pml expression in mesenchymal stem cells.

According to the International Society for Cellular Therapy (ISCT), mesenchymal stem cells are defined partially based on their capacity to form colony-forming fibroblast units (CFU-F) [24–26]. All CFU-F activity in the bone marrow was reported to be targeted in Prx1-Cre/TdTomato mice [27]. Cells targeted in those mice are heterogeneous and reside within distinct bone marrow niches within the bone marrow [28]. Interestingly, recent work has shown that deletion of specific cytokines from mesenchymal stem cells in separate vascular beds in the bone marrow, arterioles, and sinusoids, affects differently hematopoietic stem cells in these niches [11]. It remains unknown whether the arteriolar niche cells are targeted in Prx1-Cre/Pml-floxed mice. Thus, multiple questions arise from this study. Guarnerio and colleagues indicate that in Prx1-Cre/Pml-floxed mice, normal hematopoietic stem cells are not affected [13]. If arteriolar cells are not targeted in Prx1-Cre mice, does deletion of Pml in arteriolar niche cells influence normal hematopoietic stem cells? Myh11-CreER driver could be used for this purpose [11]. On the other hand, if arteriolar cells are targeted in Prx1-Cre mice, future studies will need to explore which cell populations are important for leukemic cell maintenance via expression of Pml. Are arteriolar and sinusoid niches participating in a similar manner?

### Effect of Pml deletion on mesenchymal stem cells differentiation capacity

Bone marrow mesenchymal stem cells are multipotent cells, being able to differentiate into a variety of other cell types during development, such as osteoblasts, chondrocytes, and fat cells [9, 11, 12]. Guarnerio and colleagues described that



**Fig. 1** Pml role in mesenchymal stem cells in the leukemia microenvironment. Bone marrow microenvironment is critical for the maintenance and expansion of leukemic cells. The study of Guarnerio and colleagues now suggests that mesenchymal stem cells' control of leukemic cells in the bone marrow niche is dependent on Pml expression [13]. Pml expression in mesenchymal stem cells induces the production of pro-inflammatory molecules, including CXCL1 and IL6, in

the leukemia microenvironment. Genetic deletion of Pml in bone marrow mesenchymal stem cells increased survival of mice under chemotherapeutic treatment after transplantation with leukemic cells. With the appearance of state-of-art technologies, future studies will reveal in detail the cellular and molecular mechanisms involved in leukemia progression in the bone marrow niche

upon Pml deletion, mesenchymal stem cells do not change their differentiation capacity *in vitro* after exposure to special factors [13]. Nevertheless, cell culture system may cause alterations in the cultured cells, leading them to behave differently than the same cells *in vivo*. These artificial conditions and high concentration of mitogens can induce differentiation ability in the cultured cells that may not be shared by the corresponding endogenous mesenchymal stem cells *in vivo* after Pml deletion [29]. Therefore, future studies should thoroughly explore the differentiation capacity of mesenchymal stem cells *in vivo* in Prx1-Cre/Pml-floxed mice. Mesenchymal stem cells expand upon Pml loss in the bone marrow [13]. As those cells originate osteocytes, chondrocytes, and adipocytes during development, and if the

differentiation capacity of mesenchymal stem cells is not altered, we expect that the numbers of cells derived from mesenchymal stem cells will be increased as well. Therefore, these other cell types, at a higher number, may also affect the development of leukemia seen in Prx1-Cre/Pml-floxed mice. Thus, future studies should clarify the role of Pml in mesenchymal stem cell biology in the bone marrow.

### Pro-inflammatory role of Pml from mesenchymal stem cells

Inflammation is a physiological process which happens in response to tissue stressors, generating several inflammatory mediators, including chemokines and cytokines

[30]. Multiple studies have reported that chronic inflammation elevates the risk for progression of certain types of cancer, and that the inflamed microenvironment facilitates cancer development via modulation of expression of growth factors and suppression of the immune response [31, 32]. Guarnerio and colleagues suggest, based on ELISA immunoassays, that Pml expression induces mesenchymal stem cells to produce various pro-inflammatory molecules [13]. Elegant co-culture systems between mesenchymal stem cells from Pml knockout mice and leukemic cells show that CXCL1 and IL6 derived from mesenchymal stem cells are important for the maintenance of leukemic cells. Nevertheless, CXCL1 and IL6 have not been conditionally deleted from bone marrow mesenchymal stem cells, so there is no direct evidence that mesenchymal stem cells are the only/main functionally important source of these factors. This may be addressed by analyzing the effect of genetic ablation of mesenchymal stem cells from leukemic bone marrow by using Prx1-Cre/iDTR mice [11]. Also, the generation of CXCL1-floxed and IL6-floxed mice to be crossed with mesenchymal stem cell-specific Cre drivers, such as Prx1-Cre mice [21], will allow to specifically delete CXCL1 and IL6 from mesenchymal stem cells. In addition to studies in genetic mouse models, transcriptomic and single bone marrow mesenchymal stem cell analysis, such as RNA sequencing upon Pml deletion, represent fundamental tools that will help to understand the roles of mesenchymal stem cells within the bone marrow leukemic microenvironment.

### Bone marrow heterogeneity

The bone marrow consists of a variety of cell populations among hematopoietic and non-hematopoietic cells, including mesenchymal stem cells, osteoblasts, endothelial cells, pericytes, smooth muscle cells, fibroblasts, adipocytes, glial cells, macrophages, osteoclasts, megakaryocytes, lymphocytes, and neutrophils [8, 33–57]. Several of those cellular subtypes within the bone marrow microenvironment contribute to the complex regulation of hematopoietic stem cells function and may contribute to leukemic cells maintenance as well [58]. It will be interesting to explore in future studies what is the role of Pml expressed by other cell types, besides mesenchymal stem cells, in the regulation of normal and leukemic stem cells function. Moreover, recently, it was revealed that leukemic cells create changes in the bone marrow nerves that affect the activity of mesenchymal stem cells [59–61]. Since Pml may be expressed in the nervous system [62], whether Pml expression affects nerves in leukemic settings should be examined in future studies.

### Clinical relevance

Leukemias are heterogeneous and are classified based on their lymphoid or myeloid origins, as well as on their acute or chronic progress into four common types: chronic myeloid leukemia, acute myeloid leukemia, chronic lymphocytic leukemia, and acute lymphocytic leukemia [63]. Guarnerio and colleagues report that not all leukemic models tested are affected by Pml ablation [13]. Future studies will reveal the molecular mechanisms induced by Pml deletion in mesenchymal stem cells, and why for some leukemias Pml expression in mesenchymal stem cells is not essential for their progression.

Arsenic trioxide ( $As_2O_3$ ) is a small molecular inhibitor of Pml, used for acute promyelocytic leukemia therapy, approved by the Food and Drug Administration (FDA) [64]. The authors suggest that the benefic effects of  $As_2O_3$  probably come from targeting mesenchymal stem cells as well. Nonetheless,  $As_2O_3$  has major limitations associated with toxic side effects, such as gastrointestinal reactions, skin damage, differentiation syndrome, cardiovascular defects, and liver and kidney dysfunctions [65–68]. Future works should develop Pml inhibitors that would block Pml function specifically in mesenchymal stem cells and leukemic cells to avoid side effects.

### The role of mesenchymal stem cells Pml in other cancers

In addition to leukemia, mesenchymal stem cells have been shown to play important roles in the growth and progression of other cancers, such as lymphoma [69], breast cancer [70], prostate cancer [71], head and neck carcinoma [72], colon cancer [73], gastric cancer [74], and glioblastoma [75, 76]. Also, mesenchymal stem cells may activate the motility and invasiveness of cancer cells, inducing metastatic potential in those cells [77]. It remains completely unknown what is the role of Pml in mesenchymal stem cells in the primary as well as secondary sites of other tumors outside the bone marrow. Future studies will address this question, exploring the potential of using Pml inhibitors, utilized in leukemia clinics, for other cancers.

### Conclusion

In conclusion, the study by Guarnerio and colleagues reveal that mesenchymal stem cells via Pml expression maintain leukemic cells within the bone marrow [13]. Yet, our understanding of cross-talk between different constituents of the bone marrow leukemic niche still remains limited, and the complexity of these interactions during leukemia progression should be elucidated in future studies. A big challenge that we face is how to translate animal research into humans. Improving the availability of human tissue samples may help to reach this goal.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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