ELSEVIER

Blood 142 (2023) 1333

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

506.BONE MARROW MICROENVIRONMENT

MiR-9-1 Controls Osteoblastic Regulation of Lymphopoiesis

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The highly conserved MicroRNA-9 (miR-9) family consists of three members, miR-9-1, miR-9-2 and miR-9-3. These three microRNAs are encoded by unique genes located on different chromosomes, the transcripts of which are processed into the same mature miR-9 sequence. MiR-9 is known to play a crucial role in brain development and neurogenesis, its involvement in hematopoiesis has remained uncertain. To explore this, we investigated the role of miR-9-1 in controlling hematopoiesis. Our findings revealed that miR-9-1 and miR-9-2 but not miR-9-3 were expressed in all hematopoietic cells, with higher levels observed in HSCs, MPPs, and CLPs. In order to understand the impact of miR-9 on hematopoiesis, we employed CRISPR/Cas9-mediated genome editing to create miR-9-1-deficient mice. Deletion of miR-9-1 resulted in reduced mature miR-9 expression, leading to 43% of the mice exhibiting retarded growth and postweaning lethality by 12 weeks of age. The growth-retarded miR-9-1-deficient mice experienced severe lymphopenia, primarily affecting B and T cell development. Further analysis revealed that the populations of pro/pre- and immature B cells in bone marrow (BM) were dramatically reduced in growth-retarded miR-9-1-deficient relative to wild-type control mice. The populations of peripheral T1, T2, FO and MZ B cells were also markedly reduced in growth-retarded miR-9-1-deficient relative to control mice. In addition, the numbers of DN, DP, and SP thymocytes and $CD4^+$ and $CD8^+$ T cells in the spleen were also markedly reduced in growth-retarded miR-9-1-deficient relative to control mice. Consistent with the specific impairment of lymphocyte (B and T cell) development and maturation, the growth-retarded miR-9-1-deficient mice exhibited a reduction of hematopoietic progenitors including ST-HSCs, MPPs, CMPs and especially CLPs, but had normal numbers of GMPs and MEPs. Interestingly, transplantation of miR-9-1-deficient BM cells into wild-type recipients resulted in normal lymphopoiesis, indicating that the lymphopenia was not due to hematopoietic cell autonomous effects but was caused by alterations in the BM stromal microenvironment.

We then focused on the effect of miR-9-1 deficiency on the BM niche, particularly the mesenchymal stem cell (MSC) and osteoblastic (OB) niches. The numbers of MSCs in BM stromal cells were comparable between miR-9-1-deficient and wild type mice, whereas the number of osteoblastic cells (OBs) was significantly reduced in the BM stromal cells from growth-retarded miR-9-1-deficient relative to wild-type mice. In agreement with the reduction of OBs in miR-9-1-deficient mice, the mutant MSCs failed to differentiate into OBs. Notably, miR-9-1 deficiency did not influence the differentiation of osteoclasts and adipocytes, suggesting a specific impairment in osteoblastic bone formation.

To elucidate the molecular mechanism underlying miR-9-1's regulation of osteoblastic differentiation, we conducted high-throughput RNA sequencing analysis on miR-9-1-deficient MSCs and OBs. The results revealed reduced expression of master transcription factors involved in osteoblastic differentiation, Runt-related transcription factor 2 (Runx2) and Osterix (Osx), and genes related to collagen formation, extracellular matrix organization, and cell adhesion, in miR-9-1-deficient MSCs. Additionally, we identified Follistatin (Fst) as a direct target of miR-9-1. MiR-9-1 deficiency led to increased Fst levels, which, in turn, inhibited BMP signaling in MSCs and reduced IL-7 and IGF-1.

In summary, our studies demonstrate that miR-9-1 plays a pivotal role in controlling osteoblastic regulation of lymphopoiesis by targeting the Fst/BMP/Smad signaling axis.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-181128