# TISSUE-SPECIFIC IMPAIRMENT OF STRESS HEMOPOIESIS AND MEGAKARYOCYTOPOIESIS IN THE ABSENCE OF THE HOMEODOMAIN TRANSCRIPTION FACTOR NKX2-3

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## Introduction

Beside its several immunological roles, the spleen functions as an accessory hemopoetic tissue. This activity is restricted to the splenic red pulp, which is under the developmental influence of homeodomain transcription factor Nkx2-3. In its absence the splenic vascular pattern of the red pulp and the distribution of various macrophage subsets are severely perturbed. It is not yet known how the absence of Nkx2-3 affects the inducible splenic hemopoesis.

The aim of our work was to investigate the role of the trancription factor Nkx2-3 in the stromal regulation of splenic stress hemopoiesis after inducing acute anemia and forced megakaryocyte differentiation.

## Methods

Acute anemia was triggered by a series of blood withdrawal in experimental animals. Megakaryocytopoiesis was induced by single TPO-agonist N-plate injection. The erythroid and lymphoid territories in the bone marrow and spleen of wild type BALB/c and Nkx2-3 deficient mice were compared by using flow cytometry and quantitative morphometric analysis. The erythroid and lymphoid cells of the spleen and bone marrow were labelled with Ter-119 and FITC conjugated anti-CD45 antibodies. The megakaryocytes were labelled using IBL-17 mAb in tissue immunofluorescence. The serum erythropoietin levels were determined by sandwich-ELISA and the hematocrit level of the animals were measured by a standard procedure.

## Results

In untreated Nkx2-3 knockout (KO) group the erythroid cell frequency was higher in the bone marrow, but significantly lower in the spleen, compared to the wild type control group. Upon bleeding-induced anemia, the number of erythroid cells increased in the bone marrow and spleen of BALB/c mice. In Nkx2-3 KO mice erythroid expansion was only observed in the bone marrow, while in their spleen the Ter119 positive erythroid compartment further decreased. The serum erythropoietin level increased significantly in both groups and the highest level was measured in the anemic Nkx2-3 knockout group. Similarly, while in wild-type BALB/c mice both the bone marrow and spleen could be induced to increase their megakaryocyte content by N-plate treatment, in Nkx2-3 KO mice the spleen was resistant to N-plate exposure.

## Conclusions

According to these results, homeodomain transcription factor Nkx2-3 in a tissue-specific manner plays a crucial role in the development of splenic stromal microenvironment necessary for supporting the extramedullary hematopoiesis.

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