

## Inhibition of TGF- $\beta$ 1 signaling restores both microenvironmental and stem cells abnormalities in the Gata-1<sup>low</sup> Mouse Model of Myelofibrosis

L. Sancillo<sup>1</sup>, M. Zingariello<sup>2</sup>, B. Ghinassi<sup>1</sup>, D. Bosco<sup>3</sup>, A.R. Migliaccio<sup>4</sup> and R.A. Rana<sup>1</sup>

<sup>1</sup>DMSI, Section of Human Morphology, University "G. d'Annunzio", Chieti-Pescara, Italy

<sup>2</sup>Campus Biomedico, Faculty of Medicine, Rome, Italy

<sup>3</sup>IGM CNR, Chieti, Italy

<sup>4</sup>Mount Sinai School of Medicine, NY, USA

Primary myelofibrosis (PMF) is characterized by abnormal megakaryocyte (Mk) development, fibrosis and ineffective hematopoiesis in the marrow and hematopoiesis in extramedullary sites [1]. Studies in animal models have suggested that fibrosis is established by fibroblasts activated by TGF- $\beta$ 1 released by the abnormal Mk. Increased levels of TGF- $\beta$ 1 expression in Mk have been implicated in the development of PMF. To clarify whether TGF- $\beta$ 1 alterations are involved in the development of PMF in Gata1<sup>low</sup> mice, the TGF- $\beta$ 1 content of Mk from the marrow and spleen from PMF patients and Gata1<sup>low</sup> mice was compared, the TGF- $\beta$ 1 pathway of the marrow and spleen of the Gata1<sup>low</sup> mouse PMF model was profiled and the consequences of pharmacological inhibition of TGF- $\beta$ 1 signaling, obtained through treatment with *SB431542*, was determined. Bone marrow (BM) sections from PMF patients contain 4-times more Mk than those from normal donors and great numbers of Mk are also detectable in their spleen. In addition, Mk from both BM and spleen of PMF patients reacted 34-times more intensely than normal Mk with the TGF- $\beta$ 1 antibody. Similarly the number of Mk in BM and spleen of Gata-1<sup>low</sup> mice was 2-3-fold greater than normal and these cells reacted 3-8-times more intensely with the TGF- $\beta$ 1 antibody than wild-type(wt)Mk. These results were confirmed by immunoelectron-microscopy. On average, one Mk from wild-type and Gata1<sup>low</sup> mice contained 10.3 $\pm$ 2.2 and 54.3 $\pm$ 6.5 immunogold-particles per area ( $p < 0.01$ ). *SB431542*-treatment reduced the intensity of TGF- $\beta$ 1 staining of Gata1<sup>low</sup> Mk both in BM (5.3 $\pm$ 1.1) and spleen (9.2 $\pm$ 0.7) compared to Mk both in BM (18.9 $\pm$ 0.8) and spleen (24.3 $\pm$ 0.9) of Gata-1<sup>low</sup> vehicle-treated mice, while had modest effects on the expression of VEGF and CXCL12. Inhibition of TGF- $\beta$ 1 signaling activates hematopoiesis in BM while reducing extramedullary hematopoiesis in spleen of Gata-1<sup>low</sup> mice. In addition, it reduced fibrosis, vessel microdensity, increases Ptl counts and decreases WBC and poikilocytes in the blood of Gata1<sup>low</sup> mice suggesting a potential benefit for treatments targeting microenvironment abnormalities in PMF.

### References

[1] Tefferi A. Myelofibrosis with myeloid metaplasia. *N Engl J Med.* 2000;342(17):1255-1265.

Keywords: Myelofibrosis, megakaryocytes, TGF- $\beta$ 1, hematopoiesis.