

## Thrombotic events in models GATA-1 low myelofibrosis characterized by altered localization of P-selectin during megakaryocyte development

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Patients with primary myelofibrosis (PMF) have increased risk for bleeding and thrombosis. It is debated whether propensity to thrombosis is due to increased numbers of platelet microparticles and/or to pathological platelet-neutrophil interactions. These interactions are mediated by P-selectin and even though the megakaryocytes (Mk) of MF patients express normal levels of P-selectin, it remains abnormally localized to the dense microvesicles rather than being assembled into the  $\alpha$ -granules in platelets. Mice carrying the hypomorphic *Gata1<sup>low</sup>* mutation express the same Mk abnormalities presented by PMF patients, including abnormal P-selectin localization to the DMS and develop with age myelofibrosis, that closely resembles human PMF. The aim of this study was to determine whether *Gata1<sup>low</sup>* mice would develop thrombosis with age and, in this case, the role played by P-selectin in the development of the trait. To this aim, *Gata1<sup>low</sup>* mice were crossed with *P-sel<sup>null</sup>* mice according to standard genetic protocols and *Gata1<sup>low</sup>P-sel<sup>WT</sup>*, *Gata1<sup>low</sup>P-sel<sup>null</sup>* and *Gata1<sup>WT</sup>P-sel<sup>null</sup>* or *Gata1<sup>WT</sup>P-sel<sup>WT</sup>* littermates obtained. Platelet count, hematocrit as well as platelet microparticle levels were determined on all the different mutants. It was shown that platelet counts are reduced in *Gata1<sup>low</sup>* mice. Moreover, platelet microparticles are reduced in *Gata1<sup>low</sup>* mice and P-selectin positive platelet microparticles were not found. The presence of thrombosis was determined by immunohistological staining of organs. *Gata1<sup>low</sup>* mice with or without the P-selectin null trait had a prolonged bleeding time and thrombosis was seen in adult and old *Gata1<sup>low</sup>* mice, but the *Gata1<sup>low</sup>/P-sel<sup>null</sup>* mice were rescued. Thus, presence of the P-selectin null trait rescued *Gata1<sup>low</sup>* mice from the thrombotic phenotype, but did not change level of platelet microparticles. All these data indicate that abnormal localization of P-selectin, induced by the *Gata1<sup>low</sup>* mutation, and thus, increased pathological interactions with leucocytes, is responsible for the increased presence of thrombosis seen in these mice.

Key words

Myelofibrosis, Megakaryocytes, P-Selectin.