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Deregulated Genes in Hematopoietic Stem Cells Isolated from Spleen of Patients with Myelofibrosis

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An important issue in myeloproliferative diseases research is to test the hypothesis that in the neo-angiogenesis, which is observed in the spleen and bone marrow of patients, involves endothelial cells derived from the neoplastic clone. In this study we analyzed the gene expression profile of 138 genes in CD34+ hematopoietic cells from the spleen and peripheral blood of patients with myelofibrosis (MF) and healthy individuals (HI). These genes have been selected among those that characterize the neoangiogenic gene signature or belonging to deregulated expression pathways in MF, and are listed in Table1.

By using quantitative reverse transcription-PCR (RT-qPCR), we measured the expression levels of 141 targets and 5 reference genes in CD34+ cells isolated by immunomagnetic method from the spleen and peripheral blood of 4 patients and 3 HI. For calculation of the statistical significance of the differences we used the t-test for unpaired samples. A p<0.05 was considered to be statistically significant. All data analyses were performed with GenEx (version 6.1, MultiD).

To characterize the differentially expressed genes obtained in each comparison, the lists of genes were analyzed through several tools, i.e. String, Enrichr and GSEA analysis. The comparison between spleen CD34+ cells and circulating CD34+ cells reveal a group of common genes in both patients and healthy donors. Those genes are significantly overexpressed in spleen. Some of the common genes are involved in the regulation of cell migration and in the development of blood vessels were significantly overexpressed in splenic CD34+ cells compared to circulating CD34+ cells. However, we observed that many of the genes implicated in the development of the vessels were underexpressed in CD34+ cells isolated from the spleen of patients compared to those of HI. The most significant upregulated genes in CD34+ splenic cells from patients included transcripts (GATA1, HBB, TAL1, GATA2, PTGS1) belonging to the molecular signature of CD34+ cells isolated from the bone marrow (BM) of patients with chronic myeloid leukemia (Diaz-Blanco E. et al., Leukemia 2007). Another group of upregulated genes (GATA1, TAL1, ITGB3, GATA2, PF4) are also identified in a study designed to characterize the genes essential to the development of megakaryocytes (Tenedini et al. Blood 2004). These two groups of genes are part of an expression pattern characteristic for immature stem cells as well as megakaryocyte-erythrocyte progenitor cells. Another group of genes overexpressed in patients spleens (CD34, ANGPT1, PF4, GATA2, PTGS1), which includes some of the above mentioned genes, has been observed in a comparison between circulating CD34+ cells from patients with myelofibrosis, and CD34 + cells isolated from the bone marrow of HI (Guglielmelli P. et al. Stem Cells 2007). Among the underexpressed genes in the spleen of patients we mention CXCR4, in keeping with previous observations. Interestingly, among the aforementioned genes, GATA1 and GATA2 genes are overexpressed in granulocytes of patients with MPNs regardless of the JAK2/CALR mutational status whereas PTGS1 is overexpressed in the JAK2-V617F homozygous and CALR-mutated granulocytes (Rampal et al. Blood 2014).

We focused our attention on PTGS1 because its well-known activity in regulating angiogenesis, which can be inhibited in vitro by treatment with aspirin (Tsujii, M. et al. Cell 1998). To explain the mechanism of action of low-doses aspirin in this context, it has been proposed a model that involves permanent inactivation of PTGS1 in platelets. Indeed, PTGS1 is the only cyclooxygenase isoenzyme present in platelets. Moreover, Dixon and colleagues (Dixon, D.A. et al. JCI 2006), demonstrated that activated platelets induced the expression of PTGS2 in monocytes. It is believed that the synthesis of prostaglandin E2 by PTGS2 in tissues, is linked to an increment of angiogenesis and cell proliferation, and to a reduction of apoptosis. According to this concept, our data point toward a model in which, in the spleen of patients with MF, an altered hematopoietic stem cell differentiation could induce an inflammation-mediated angiogenesis through the overexpression of PTGS1 and a consequent induction of PTGS2 in cells of the splenic microenvironment.
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