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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 heathy 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 under-expressed 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.

Gene symbol	Refseq ID	Gene symbol	Refseq ID	Gene symbol	Refseq ID	Gene symbol	Refseq ID
ACE	NM_000789	EFNB2	NM_004093	IL6	NM_000600	PROK2	NM_021935
ACTA2	NM_001141945	EGF	NM_001178130	IL8	NM_000584	PROM1	NM_001145847
AKT1	NM_005163	EGFR	NM_005228	ITGAV	NM_001144999	PTGS1	NM_000962
ANG	NM_001097577	ENG	NM_000118	ITGB3	NM_000212	RUNX1	NM_001001890
ANGPT1	NM_001146	EPAS1	NM_001430	JAG1	NM_000214	S1PR1	NM_001400
ANGPT2	NM_001118887	EPHA2	NM_004431	JAK2	NM_004972	SERPINE1	NM_000602
ANGPTL4	NM_001039667	EPHB4	NM_004444	KDR	NM_002253	SERPINF1	NM_002615
ANPEP	NM_001150	ERBB2	NM_004448	KRAS	NM_004985	SMAD2	NM_001003652
ATXN1	NM_000332	ESM1	NM_007036	LAMC2	NM_005562	SMAD3	NM_001145102
B2M	NM_004048	ETS1	NM_001143820	LECT1	NM_001011705	SPHK1	NM_001142601
BAI1	NM_001702	F3	NM_001993	LEP	NM_000230	SPI1	NM_001080547
BMP2	NM_001200	FGF1	NM_000800	LMO2	NM_001142315	SPINT2	NM_001166103
BMP4	NM_001202	FGF2	NM_002006	LOX	NM_001178102	TAL1	NM_003189
CCL2	NM_002982	FGFR1	NM_023110	LOXL1	NM_005576	TEK	NM_000459
CD33	NM_001082618	FGFR3	NM_0001402	LOXL2	NM_002318	TFPI	NM_006287
CD34	NM_001025109	FIGF	NM_004469	LOXL3	NM_032603	TFPI2	NM_006528
CD38	NM_001775	FLI1	NM_002017	LOXL4	NM_032211	TGFA	NM_001099691
CD44	NM_000610	FLT1	NM_001159920	MDK	NM_001012333	TGFB1	NM_000660
CDH5	NM_001795	FLT4	NM_002020	MLL	NM_001197104	TGFB2	NM_001135599
COL18A1	NM_030582	FN1	NM_002026	MMP1	NM_001145938	TGFBR1	NM_004612
COL4A3	NM_000091	FUT4	NM_002033	MMP14	NM_004995	TGM2	NM_004613
CTGF	NM_001901	GAPDH	NM_002046	MMP2	NM_004530	THBS1	NM_003246
CTNNB1	NM_001904	GATA1	NM_002049	MMP9	NM_004994	THBS2	NM_003247
CXCL1	NM_001511	GATA2	NM_001145661	MYB	NM_001130172	TIE1	NM_005424
CXCL10	NM_001565	GATA3	NM_001002295	MYCN	NM_005378	TIMP1	NM_003254
CXCL12	NM_001033886	GLI1	NM_001160045	NF1	NM_000267	TIMP2	NM_003255
CXCL12_3	NM_001178134	HBB	NM_000518	NOS3	NM_000603	TIMP3	NM_000362
CXCL5	NM_002994	HGF	NM_000601	NOTCH1	NM_017617	TNF	NM_000594
CXCL6	NM_002993	HIF1A	NM_001243084	NRP1	NM_001244972	TYMP	NM_001113755
CXCL9	NM_002416	HOXB4	NM_024015	NRP2	NM_003872	UBC	NM_021009
CXCR4-1	NM_003467	HPRT1	NM_000194	OSR1	NM_145260	VEGFA	NM_001025366
CXCR4-2	NM_001008540	HPSE	NM_001098540	PECAM1	NM_000442	VEGFB	NM_001243733
CXCR4-TOT	-	ID1	NM_002165	PF4	NM_002619	VEGFC	NM_005429
DES	NM_001927	IFNG	NM_000619	PGF	NM_001207012	VWF	NM_000552
EDN1	NM_001168319	IGF1	NM_000618	PLAU	NM_001145031	WNT1	NM_005430
EDN1	NM_001168319	IKAROS	NM_001220771	PLAUR	NM_001005376	YWHAZ	NM_001135699
EFNA1	NM_182685	IL1B	NM_000576	PLG	NM_000301		

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