Differential expression of JAK2 and Src kinase genes in response to hydroxyurea treatment in polycythemia vera and essential thrombocythemia

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Abstract

Design and methods: This study investigates the differential gene expression profile of JAK2^{V617F} positive MPN patients, with and without response to HU treatment. Twenty-one PV, 28 ET, eight secondary erythrocytosis, and 30 controls were studied.

Results: Thirty-four genes were over-expressed in patients who did not respond to HU. Of these, some participate in proliferative pathways: *MAPK*, *AKT*, Src kinase (SFK), and JAK2 pathway. JAK2 allele burden was similar between groups of responders and non responder.

Conclusions: A molecular fingerprint distinguishes JAK2^{V617F} positive MPN patients without response to HU treatment, with over-expression of *JAK2*, *MAPK14*, *PIK3CA* and SFK genes.

Introduction

The discovery of the JAK2^{V617F} point mutation has become a hallmark in the diagnosis of the classic chronic Philadelphia-negative myeloproliferative neoplasms (MPN) namely, polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).[1] As a result, WHO diagnostic criteria were revised in 2007, and JAK2 mutations and MPL mutations were included as major criteria for the diagnosis of PV, ET and PMF.[2] Moreover, the JAK2^{V617F} allele burden has been included in response criteria for PV and ET by a recent European Leukaemia Net (ELN) consensus conference.[3] Despite the fact that JAK2^{V617F} has facilitated the diagnosis of MPN, and improved our knowledge on their pathogenesis, there are still a number of unanswered questions.[4]

Although formerly believed to be a single-step process, recent evidence has shown that the JAK2^{V617F} mutation is neither the sole, nor sufficient molecular event, responsible for MPN. It may also not be the initial one, and other somatic mutations may precede JAK2^{V617F}. [5-7] There is evidence of clonal heterogeneity and presence of different mutations in the same patient.[8, 9]

The current first line of treatment for PV and ET patients with a high risk of thrombosis is still hydroxyurea (HU).[10] The cytoreductive activity of HU is based on its anti-metabolic role as a cell cycle-specific analog of urea, with activity in the S-phase. HU inhibits the enzyme, ribonucleotide reductase, which converts ribonucleotides to deoxyribonucleotides (critical precursors for *de novo* DNA biosynthesis and repair). Up to 15 to 25% of patients show resistance or intolerance to HU.[11, 12] The mechanism of resistance is believed to be the increased expression of ribonucleotide reductase due to gene amplification, increased transcription, and post-transcriptional mechanisms.[13] Despite this knowledge, there is still no clear understanding of the underlying molecular mechanism and consequences of HU activity.

We aimed to analyze the differential gene expression profile of JAK2^{V617F} positive MPN patients with or without response to HU treatment, to determine possible mechanisms for a poor response. As a consequence, we hoped to identify alternative routes for targeted therapy. In addition, we aimed to correlate these results with clinical and laboratory features.

Design and methods

Patients

Forty-nine patients, diagnosed with MPN (21 PV and 28 ET) with the JAK2 V617F mutation were included in the study, as well as eight patients with secondary erythrocytosis (SE) to hypoxemia, and 30 healthy control donors. A MPN diagnosis was established on the basis of World Health Organization criteria (WHO) 2001/2008,[2] or the

Polycythemia Vera Southern Study Group (according to the standard criteria at the moment of diagnosis). Twenty MPN patients were studied at diagnosis (as control population) and 29 after treatment.

Clinical and laboratory features at diagnosis and follow-up features of the JAK2 V617F PV and ET patients were assessed and are shown in Table 1.

Response to treatment with HU in PV and ET was defined according to update recommendations [10] and to clinico-hematological response criteria for PV and ET recently published by the European Leukaemia Net (ELN) consensus conference [3].

The study was approved by the local ethics committee and written informed consent was obtained from all patients, according to the Declaration of Helsinki.

Nucleic acids extraction and JAK2^{V617F} quantification

Peripheral venous blood was collected in EDTA and immediately processed. Granulocytes were isolated by Ficoll-Paque (Sigma-Aldrich, Saint Louis, Mi USA). density gradient centrifugation, as previously described. Erythrocytes were eliminated by lysis with a commercial buffer (Red Blood Lysis Buffer, from Roche Applied Sciences, Mannheim, Germany). DNA was extracted using the Maxwell 16 SEV automated extraction system (Promega co., Madison, Wi, USA). mRNA was extracted from 1×10^6 cells, with TRIzol(R) Reagent (Invitrogen Life Technologies, Inc., Paisley, UK) according to the manufacturer's instructions, with more than 90% granulocytes. Lymphocyte contamination was assessed in five samples by flow cytometry, and was less than 2% of the total cell count. DNA and RNA quantity and quality were assessed on a ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) for low density array experiments, and by nanoelectrophoresis using the Nano lab-on-a-chip assay for total eukaryotic RNA (Bioanalyzer, Agilent Technologies, Palo Alto, CA, USA) for microarrays. Retrotranscription was performed with a MMLV-modified enzyme, according to manufacturer's instructions (Applied Biosystems, Palo Alto, CA, USA). Mutational screening for JAK2^{V617F} was performed with real-time (RT) PCR using DNA from whole peripheral blood from the study sample.[14, 15]

Microarray analyses

As screening proceeding fourteen samples from JAK2^{V617F} positive patients diagnosed with MPN (three PV and 11 ET) were included in the study, as well as 20 samples from healthy control donors (in four pools from five samples); 11 MPN were under treatment and 3 were under no treatment. Microarray expression profiles were obtained using the Whole Human Genome Oligo Microarray Kit (Agilent Technologies, Inc., Santa Clara, CA, USA). Hybridization was performed with Stratagene Universal Human Reference RNA, and a mix of tumor cell lines (Agilent Technologies Stratagene, Inc., Santa Clara, CA, USA). Briefly, for each sample 2 μ g of total RNA was mixed with 2 μ L of a 5,000-

fold dilution of Agilent's Two-Color Spike-in RNA control. The mixture was amplified using the low-input RNA amplification kit (Agilent Technologies Inc., Santa Clara, CA, USA). Following amplification and labeling with Cy3, each sample was assessed on the Nanodrop ND-1000 (NanoDrop Technologies, Wilmington, DE, USA) to measure yield and specific activity. The amplified and labeled samples were hybridized in a rotating oven to Agilent 44K human whole genome microarrays, according to the manufacturer's instructions. The images were scanned with a G2565BA Microarray Scanner System (Agilent Technologies Inc., Santa Clara, CA, USA). The data were processed and normalized with the use of Feature Extraction (v.9.0) software. Each time point was additionally normalized to time zero. Genes with more than 10% missing values were discarded. Normalized log₂ ratios were scaled between arrays to make all data comparable, and processed again with pre-processor of GEPAS 3.1 (<http://gepas3.bioinfo.cipf.es/>) to purify the data.

The student's test was employed as statistical tool in the gene expression pattern analysis suite (GEPAS 3.1), Pomelo II (<http://pomelo2.bioinfo.cnio.es/>) and gene set enrichment analysis (GSEA) programs, used to interpret gene expression data and to determine statistical significance of differential gene expression, as described.[16]

The supervised classification of healthy control donors, ET and PV samples into categories was performed.

Low-density quantitative array (LDA)

To reassess microarray data, forty JAK2^{V617F} positive patients, diagnosed with MPN were included in the study: 20 MPN patients receiving HU treatment (10 PV, and 10 ET) and 20 MPN newly diagnosed patients (10 PV, and 10 ET), used as control population to assay the influence of phenotype. Eight secondary erythrocytosis (SE) to hypoxemia as well as 10 healthy control donors (in two pools from five samples) were also included in the study. Related to the 20 MPN patients with HU treatment, nine of them showed response to HU (four PV and five ET) and 11 were non-responders (six PV and five ET). Among the MPN patients with HU treatment, two PV and three ET patients had been previously included in the microarray assay

Eighty-four genes were selected for their differential expression from several different dichotomous analyses in the microarray study. Another nine genes, though without differential expression in microarray analysis, were selected for their key role in erythropoiesis, thrombopoiesis, or signalling pathways in MPN; and three housekeeping genes (*GADPH*, *18s rRNA*, and *ABL1*) were also included (Supplementary Table 1: Supplementary data). All these genes were assayed by quantitative PCR (using the 96 TaqMan Low Density Arrays platform LDA). Besides, three

individual TaqMan assays were performed with three additional genes, of interest according to other MPN studies, in the same samples of patients.

Custom-made TaqMan low-density arrays with a setup of 96 different genes were applied for relative mRNA quantification. cDNA samples were subjected to RT-PCR in duplicate in an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Palo Alto, CA, USA). Relative expression of each gene was quantified by the comparative cycle threshold (Ct) method, using the Abelson murine leukemia viral oncogene homolog 1 (*ABL1*) as an endogenous control. Gene expression quantification was achieved using the comparative Ct method for relative quantification, in which the amount of target is expressed as $2^{-\Delta Ct}$. [15]

Statistical analyses

Statistical analyses of gene expression results in LDA, and of the clinical and biological data, and their relationship with the gene expression data, were performed using the SPSS15 software (SPSS Inc., Chicago, Illinois, USA). Differences between non-normally distributed group data were analyzed using the non-parametric Wilcoxon analysis. Statistical significance was considered when *P* was less than 0.05.

Results

Microarray results based on biological and functional pathways analyses

Microarray analysis, used as screening test, showed 84 genes with significant differential expression by GSEA (conditions required in GSEA analysis: core enrichment: YES), related to biological and functional pathways of GSEA tool (significant different expression by GSEA was considered when *P* was < 0.05 and/or the false discovery rate (FDR) was < 0.25).

Genes were selected from different analyses performed in a dichotomic manner: ET versus PV, ET versus Controls, PV versus Controls and MPN with response to HU against those without response to HU.

Low density array analysis

Gene expression profile and response to HU treatment

A set of 34 genes were found to be differentially expressed in MPN patients who either responded or showed no response to HU treatment, and were significantly over-expressed in those patients with no response (Table 2; Figure 1). Interestingly, the following genes were significantly over-expressed in patients who showed no response to HU treatment: *JAK2* (ER = 2.482 vs 1.096; *P* = 0.008), *IGF1R* (ER = 3.076 vs 2.259; *P* = 0.026), *MAPK14* (ER = 4.885

vs 3.834; $P = 0.007$), *FCRE1G* (ER = 4.760 vs 3.295; $P = 0.004$), *LYN* (ER = 5.372 vs 4.386; $P = 0.017$), *BTB* (ER = 1.121 vs 0.082; $P = 0.016$) and *PIK3CA* (ER = 4.826 vs 3.731; $P = 0.011$) (Figure 1).

JAK2 was over-expressed in patients who did not respond to treatment; and in patients who did respond to treatment, JAK2 expression was lowered to equivalent levels to those of SE patients and healthy controls. A similar gene expression pattern was observed for *MAPK14*, *FCRE1G*, *LYN*, *BTB* and *PIK3CA* (Figure 1).

These differences were not due to disease duration because when we compared patients with a short disease duration (below two years) and patients with a long disease duration (over two years) no differences in gene expression profile were found (Supplementary Table 2, supplementary data).

The gene expression profile of the control group was similar to that of patients who responded to HU treatment.

JAK2 allele burden quantification

In order to assay if the lowering expression of JAK2 gene was due to a decrease of the JAK2^{V617F} allele burden, it was measured in patients at diagnosis and under HU treatment. When measured after HU treatment, JAK2^{V617F} allele burden diminished in PV, but only in a significant manner in ET ($P = 0.003$). However, the allele burden differences between patients who responded, or those who did not respond to HU, were not statistically significant (Figure 1).

Gene expression profile associated with response is not influenced by phenotypic diversity

To assay whether the different gene expression profile between responders to HU treatment and the non responder group could be due to phenotypical differences, gene expression of patients under HU treatment was compared with a balanced population of patients at diagnosis.

Upon separate analysis of samples from PV and ET patients receiving HU treatment, and those patients at diagnosis, only one gene, *MMP14*, was over-expressed in PV newly diagnosed patients compared to ET at diagnosis. Moreover, this over-expression was maintained when comparison was established between treated and untreated ET patients against PV treated and untreated (ER: expression ratios = -4.442 vs -2.794; $P = 0.000$) (Table 2; Figure 2).

Correlation between clinical and laboratory features and gene expression profile in PV and ET

Clinical and laboratory variables, referred in Table 1 were analyzed and compared in a dichotomic manner with gene expression profile, to search correlates: (1) at diagnosis: gender, age, splenomegaly, hepatomegaly, hemoglobin, hematocrit, white blood cells, platelets, lactate dehydrogenase, thrombotic events, hemorrhagic events, JAK2^{V617F} allele burden; (2) and in the follow up: disease duration, treatment duration, thrombotic events after diagnosis, hemorrhagic events after diagnosis, and response to HU treatment (Supplementary Table 2: Supplementary data).

Discussion

Microarray analysis looking for a differential gene fingerprint profile between JAK2^{V617F} MPN different groups treated with HU resulted in the identification of 84 genes with significant differential expression. These genes play key roles as transcription factors (*LYN*, *PIK3CA*), in MAP kinase (MAPK) signaling pathways (*MAPK14*, *RAF1*, *RASA1*, *YWHAH*), as tyrosine kinases (*JAK2*, *BTK*), in development and function of the hematological system (*CD44*, *MAP1B*, *JARID2*, *HMGB2*, *IKZF1*), and in other signaling and apoptotic pathways (*SKAP2*, *PLAUR*, *LRMP*). Some of these genes have been previously outlined in other microarray analyses or functional studies. [17, 18]

When we looked at this gene signature with LDA (a real time PCR approach), we observed a differential gene expression profile in MPN patients according to response to HU. We found 34 genes that were over-expressed in patients who did not respond to HU treatment. Interestingly, among these 34 over-expressed genes, some participate in four important proliferative pathways: *MAPK14*, *MAP2K1*, *RAF1* (MAPK pathway), *PIK3CA*, *FRMD4B* (AKT pathway), *LYN*, *FCER1G*, *BTK*, *SKAP2* (Src family kinase, SFK), and *JAK2*, *IGFRI* (JAK2 pathway). Gene expression did not change with disease evolution but it did with the response to treatment.

A number of genes in the MAPK pathway were differentially expressed in MPN patients relative to their response to HU. An over-expression of genes implicated in this pathway was shown in the group who did not respond to HU, notably *RAF1*, *MAP2K1* and *MAPK14*. In the group of patients who did respond to HU treatment, MAPK genes expression was lowered to levels equivalent to those observed in SE patients and controls. The activation and over-expression of *MAPK14* has been described in PV pathogenesis.[1, 17, 19] Over-expression of genes in the MAPK pathway may be involved in the lack of response to HU treatment, although by yet unknown mechanisms, and probably related to *JAK2* gene expression.

The *AKT* gene, with a crucial role in the AKT pathway, has been widely implicated in the pathogenesis of MPN (PV and ET), by over-activation and over-expression.[1, 19, 20] We found that the *PIK3CA* gene, an AKT regulator, was also over-expressed in patients who did not respond to HU treatment. This over-expression may also be involved in the lack of response to treatment, and correlate with *JAK2* over-expression.

The SFK are also suggested to contribute to signalling by EpoR,[21] although they seem not to be indispensable for the PV phenotype induced by JAK2^{V617F}, as shown by bone marrow reconstitution studies in mice deficient for *LYN*, *FYN* or *Fgr* kinases.[22] Our results showed that *BTK* and other SFK members, such as *LYN*, *FCER1G* and *SKAP2*, were over-expressed in patients who did not respond to HU treatment.. Thus, an over-expression of these genes may be related to a lack of response to HU, while decreased levels, similar to those observed in SE patients and controls, may be related to a good response to HU treatment. These findings could be of great interest, since those

patients with over-expressed SFK genes may benefit from treatment with SFK inhibitors (such as Dasatinib). In fact, some groups are testing Dasatinib in cell cultures from PV patients with some success.[22, 23]

In addition, HU treatment in MPN patients showed a resulting decrease in, not only the *JAK2*^{V617F} allele burden, but also in response to treatment in *JAK2* gene expression. Interestingly, while *JAK2* was over-expressed in patients who did not respond to treatment, in those patients who responded well to HU, *JAK2* gene expression was lowered to levels equivalent to those of SE patients and healthy controls. We also showed that, although the *JAK2*^{V617F} allele burden diminished after HU treatment, as reported by others,[24] there was no statistical difference between the levels of the *JAK2*^{V617F} allele burden in our different patient groups, with or without a response to HU. However, *JAK2* gene differential expression between the same groups of patients with or without a response to HU treatment was statistically significant. Thus, *JAK2* over-expression may reflect of lack of response to HU, although the mechanism for this is unknown. The criteria for a lack of response to HU treatment could thus be expressed, either by the mutant allele burden, and/or by *JAK2* over-expression. To our knowledge, this is the first study to show these results, and could be useful as an additional parameter of response.

Finally we found that this different pattern of expression was not due to phenotypic differences, as demonstrated when compared with a control group of patients without treatment.

Only one gene, *MMP14*, remained significant between PV and ET patients at diagnosis. *MMP14* is a matrix metalloprotease involved, among other things, in tumor metastasis. It has an indirect connection with some proliferative pathways, such as the MAPK pathway. Some authors have made reference to the importance of the MAPK pathway in PV pathogenesis, and its connection with *JAK2*. [1, 17] On the other hand, *MMP14* has also been demonstrated to be over-expressed in PMF, and is modulated by *TIMP2*, which has erythroid-potentiating activity.[25, 26] Thus, the over-expression of *MMP14* in PV, compared with ET, may contribute to the final myeloproliferative phenotype of PV by enhancing the commitment to erythropoiesis via MAPK pathway, either by JAK/STAT, *TIMP2* or by alternative routes.

To conclude, our results suggest that a differential molecular fingerprint of genes distinguishes MPN patients who either do or do not respond to HU treatment. *JAK2*, *MAPK14*, *PIK3CA* and SFK genes are over-expressed in patients who do not respond to HU. In our group of patients the *JAK2*^{V617F} allele burden decreased with HU treatment, however a significant diminution was not observed in response to treatment. On the other hand, patients with no response to treatment over-expressed *JAK2*. These results need to be confirmed with further functional studies.

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Table 1. The clinical and laboratory features of JAK2^{V617F} positive PV and ET patients at diagnosis and follow-up. PV, polycythemia vera; ET, essential thrombocythemia; HU, hydroxyurea; M, male; F, female; WBC, white blood cells, LDH, lactate dehydrogenase NA: not applicable; a. Median value (range) is reported.

Table 2. Differential gene expression of JAK2^{V617F} positive polycythemia vera (PV) and essential thrombocythemia (ET) patients. Results of the low density array (LDA) analysis ($P < 0.05$) and results of Wilcoxon analyses between clinical and laboratory features relative to the gene expression profile are shown.

Table 1. The clinical and laboratory features of JAK2^{V617F} positive PV and ET patients at diagnosis and follow-up.

Clinical and laboratory variables	PV with treatment	ET with treatment	PV at diagnosis	ET at diagnosis
Patients	10	16	11	12
Gender (M/F)	5/5	6/10	6/5	2/10
Age at diagnosis ^a	64.5 (37-77)	65 (20-92)	57 (21-74)	56 (39-87)
Splenomegaly at diagnosis	0/10	0/16	1/11	1/12
Hepatomegaly at diagnosis	2/10	2/16	1/11	0/12
Hemoglobin (g/100 mL) at diagnosis ^a	20.05 (17.5-22.5)	15.2 (12.5-16.4)	15.5 (14.2-21.3)	13.9 (11..9-19.5)
Hematocrit at diagnosis ^a	58 (51.1-66.4)	45.9 (38.5-49.4)	46.3 (42.7-61)	43 (39.2-59)
WBC (x10 ⁹ /L) at diagnosis ^a	11.1 (5.91-14.7)	9.61 (5.96-19.2)	8.44 (5.2-14.34)	10.2 (6.9-14.8)
Platelets (x10 ⁹ /L) at diagnosis ^a	436.5 (305-1328)	821 (552-11580)	582 (215-819)	655 (538-996)
Increased LDH at diagnosis	4/10	3/16	1/11	1/2
Thrombotic events at diagnosis	0/10	3/16	2/11	1/12
Hemorrhagic events at diagnosis	0/10	2/16	0/11	0/12
Thrombotic events after diagnosis	4/10	3/16	-	-
Hemorrhagic events after diagnosis	0/10	0/16	-	-
JAK2V617F allele burden ^a	20.53% (1.64-100)	6.95% (0.67-29.52)	36.84% (2.32-128.87)	23.45% (2.32-36.09)
Disease duration in months	60.5 (1-122)	118 (4-216)	0	0
Treatment duration in months ^a	60.5 (1-122)	118 (4-216)	0	0
Response to HU treatment	4/10	7/16	-	-

Table 2. Differential gene expression of JAK2^{V617F} positive polycythemia vera (PV) and essential thrombocythemia (ET) patients.

Genes	ET vs PV			ET vs PV (with HU treatment)			ET vs PV (at diagnosis)			Response to HU Treatment		
	(P-value)		(ratio)	(P-value)		(ratio)	(P-value)		(ratio)	(P-value)		(ratio)
	ET	PV		ET	PV		ET	PV		Yes	No	
patients	20/20.	20	20	10/10.	10	10	10/10.	10	10	11/9.	11	9
ACSL	0.044*	4.072	4.609	0.000*	3.611	5.602	0.598	4.534	3.617	0.017*	4.235	5.060
ANXA1	0.040*	2.282	2.756	0.000*	1.953	3.275	0.975	2.610	2.209	0.020*	2.320	2.973
BTK	0.0032*	0.467	1.636	0.000*	-0.707	1.807	0.729	1.642	1.466	0.016*	0.082	1.121
CASP8	0.003*	2.838	3.459	0.000*	2.210	3.775	1.000	3.466	3.144	0.039*	2.667	3.391
CD44	0.008*	2.422	3.229	0.000*	1.796	3.961	0.424	3.049	2.497	0.005*	2.348	3.527
CXCL1	0.054	2.359	3.156	0.000*	1.214	3.168	0.940	3.504	3.144	0.014*	1.713	2.775
EDN1	0.218	-1.292	-0.679	0.002*	-1.990	-0.433	0.598	-0.593	-0.926	0.009*	-1.818	-0.471
F2R	0.02*	-2.409	-2.308	0.091	-2.404	-1.632	0.452	-2.414	-3.019	0.019*	-2.420	-1.526
FCER1G	0.0021*	3.511	4.682	0.000*	2.494	5.415	0.497	4.527	3.950	0.004*	3.295	4.760
FRMD4B	0.001*	1.148	2.579	0.000*	0.104	2.931	0.845	2.191	2.228	0.001*	0.735	2.473
GAPDH	0.011*	2.464	3.167	0.000*	1.719	4.164	0.577	3.208	2.170	0.037*	2.520	3.457
GSK3B	0.094	1.550	2.191	0.000*	0.567	2.650	0.220	2.533	1.733	0.024*	1.176	2.137
HMGB2	0.014*	3.188	3.732	0.000*	2.185	4.375	0.869	4.190	3.089	0.014*	2.754	3.924
IGF1R	0.052	2.417	2.703	0.000*	1.715	3.538	0.798	3.120	1.867	0.026*	2.259	3.076
IKZF1	0.022*	1.102	2.187	0.000*	0.503	2.190	0.752	1.700	2.184	0.024*	0.970	1.807
ITGAM	0.065	2.718	3.384	0.000*	1.905	3.912	0.283	3.532	2.857	0.004*	2.400	3.530
JAK2	0.002*	1.527	2.862	0.000*	0.444	2.995	0.892	2.611	2.729	0.008*	1.096	2.482
LITAF	0.002*	4.366	5.142	0.000*	4.146	6.122	0.497	4.586	4.161	0.016*	4.693	5.673
LRMP	0.007*	2.496	3.532	0.000*	1.558	3.740	0.752	3.434	3.324	0.001*	2.134	3.280
LYN	0.002*	4.535	5.686	0.000*	0.648	1.931	0.616	5.321	5.450	0.017*	4.386	5.372
MAP2K1	0.053	1.070	1.683	0.001*	1.931	0.647	0.598	1.493	1.435	0.045*	0.972	1.677
MAPK14	0.006*	3.606	4.314	0.000*	3.200	5.414	0.460	4.012	3.215	0.007*	3.834	4.885
MMP14	0.000*	-4.422	-2.794	0.001*	-4.430	-2.759	0.003*	-4.414	-2.829	0.106	-3.995	-3.105
PECAM1	0.021	2.641	3.562	0.000*	1.676	3.782	0.869	3.605	3.342	0.030*	2.414	3.114
PIK3CA	0.015*	-0.490	0.217	0.000*	-1.424	0.434	0.752	0.445	0.000	0.020*	-0.881	-0.024
PLA2G4A	0.082	-1.904	-1.446	0.002*	-2.609	-1.301	0.798	-1.199	-1.590	0.022*	-2.289	-1.547
RAF1	0.005*	2.039	2.954	0.000*	1.352	3.082	0.845	2.726	2.826	0.003*	1.811	2.713
S100A4	0.059	3.779	4.334	0.000*	3.072	4.845	0.478	4.486	3.823	0.037*	3.679	4.301
SEC31B	0.114	-0.260	0.194	0.002*	-0.463	0.750	0.346	-0.058	-0.391	0.026*	-0.184	0.544
SELL	0.005*	3.681	4.666	0.000*	2.765	5.148	0.892	4.596	4.184	0.014*	3.527	4.482
SELP	0.010*	-1.000	0.040	0.002*	-1.635	0.166	0.641	-0.365	-0.086	0.019*	-1.067	-0.327
SKAP2	0.008*	2.230	3.254	0.000*	1.330	3.675	0.845	3.131	2.832	0.013*	2.053	3.052
SPON1	0.469	-5.401	-5.314	0.030*	-5.398	-4.389	0.459	-5.403	-6.239	0.027*	-5.269	-4.435
TAP1	0.072	2.677	3.522	0.000*	2.441	3.923	0.684	2.913	3.121	0.042*	2.921	3.500
YWHAH	0.025*	1.650	2.436	0.000*	1.188	2.937	0.660	2.137	1.880	0.034*	1.672	2.538

Figure 1 The differential gene expression profile in polycythemia vera (PV) and essential thrombocythemia (ET) patients relative to their response to hydroxyurea (HU) treatment. Box plot of *JAK2*, *MAPK14*, *PIK3CA* and *FCER1G* expression ratios: Y axis: ratio; X axis: in every box plot compared groups: healthy controls, SE, PV and ET.

Figure 2 *MMP14* gene is differentially expressed in *JAK2*^{V617F} positive polycythemia vera (PV) and essential thrombocythemia (ET) patients at diagnosis. Box plot of *MMP14* expression ratios: Y-axis: *MMP14* ratios; X-axis: from left to right: healthy controls, SE, PV and ET patients.