Aims: To evaluate the effect of JAK inhibitors on ABCB1, ABCG2, SLC22A1 and SLCO1A2 mRNA expression in Caco-2, HepG2 and SET-2 cell lines.
Methods: Caco-2, HepG2 and SET-2 (JAK2V617F positive) cell lines were treated with 0 to 4 μM of *JAK Inhibitor I* (Merck/Calbiochem®, Darmstadt, Germany), a commercial ATP-competitive JAKs inhibitor, for 24 hours. After treatment, Caco-2 and HepG2 cells were submitted to viability and DNA fragmentation tests. JAK-STAT pathway inhibition was verified by STAT-5 phosphorylation inhibition (western blot) in SET-2 cells. The ABCB1, ABCG2, SLC22A1 and SLCO1A2 mRNA expressions were evaluated in Caco-2, HepG2 and SET-2 treated with *JAK Inhibitor I* by real time PCR, using Taqman® Gene Expression Assays (Applied Biosystems, Foster City/CA, USA).
Results: *JAK Inhibitor I* did not affect HepG2 cell line viability or DNA fragmentation when used in concentrations of 0 to 4 μM after 24h of cells treatment (P>0.05). In Caco-2 cells, the treatment with *JAK Inhibitor I* in a concentration of 2 μM and 4 μM by 24 hours leads to cell viability loss (P<0.001), although the DNA fragmentation were similar to control (P>0.05). The gene expression was analyzed in cell lines treated with *JAK Inhibitor I* in a concentration range of 0 to 1 μM. In SET-2, the treatment with 1 μM for 24 hours leads to JAK-STAT pathway inhibition. In HepG2, treatment with *JAK Inhibitor I* did not change ABCB1 and ABCG2 mRNA expression (P>0.05). Likewise, *ABCB1*, *ABCG2*, *SLC22A1* and *SLCO1A2* expressions were similar before and after *JAK Inhibitor I* treatment in Caco-2 and SET-2 cells lines (P>0.05).
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Summary and Conclusions: Taken together, the data suggest that *JAK Inhibitor I* did not change the *ABCB1*, *ABCG2*, *SLC22A1* and *SLCO1A2 mRNA* expression in HepG2, Caco-2 and SET-2 cell lines. These findings indicate that JAK-STAT pathway inhibitors might not offer resistance potential by upregulation/downregulation of these drug transporters genes.

PB1718

GENETIC AND EPIGENETIC ABNORMALITIES IN MYELOPROLIFERATIVE NEOPLASMS
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Background: Recent studies have revealed a number of genetic and epigenetic alterations that likely contribute to disease pathogenesis and determine clinical outcome. In addition to the *JAK2 V617F* mutation, pathogenesis of myeloproliferative neoplasm (MPN) has evolved from a simple to a complex model, including a number of novel mutations. However, little is known about the frequencies and distribution patterns of genetic and epigenetic mutations in Korean population.
Aims: We investigated the frequencies and distributions of genetic and epigenetic mutations in Korean patients with MPN.
Methods: We investigated 75 patients with *BCL-ABL1* negative MPN. The *BCR-ABL1* rearrangement was assessed by reverse transcription polymerase chain reaction (RT-PCR). The *JAK2* V617F mutation was assessed by allele-specific PCR or direct DNA sequencing. We searched for mutational hot spots in *IDH1* (R132), *IDH2* (R140 and R172), *DNMT3A* (R882), *CBL* (exons 8 and 9), *EZH2* (exon 17~19), *WT1* (exons 7 and 9), *JAK2* exon 12, *MPL* (W515L), *ASXL1* (exon 13), *SH2B3* (exons 2, 7 and 8) and *SRSF2* (P95) using direct sequencing.
Results: In this study, *JAK2* V671F mutation was detected in 64% of *BCR-ABL1*-negative MPN. We found two *SH2B3* mutations in exon 8 which is recently reported mutational region in the Korean population study. Compared to the previous reports, the mutational hot spots in this study are not found except *SH2B3* (2.7%). However, we found 88% of *WT1* rs16754 and analyzed genotype-specific risks by comparing the genotype distribution. Especially, the individuals carrying mutant G alleles of *WT1* rs16754 found low prevalence of MPN for *WT1* rs16754 (Hazard ratio 0.103~0.648, p<0.05), therefore, G allele for *WT1* rs16754 might be risk reducing alleles for developing a MPN. There was no significant difference in overall survival between genotypes.
Summary and Conclusions: This study identified a very low prevalence of genetic and epigenetic mutations in the Korean patients with MPN except *SH2B3* .We observed a significant difference in allele and genotype frequencies of *WT1* rs16754 in an Asian population compared to a western population and wild allele G of *WT1* rs16754 might be a risk-reducing association for developing MPN in Korean population.

PB1719

OXIDATIVE STRESS IN MYELOPROLIFERATIVE NEOPLASMS
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Background: Oxidative stress is an invasive condition with increased reactive oxygen species, now recognized as an important characteristic of malignant disorders as well as their progression.
Aims: The aim of this study was to evaluate the role of oxidative stress induced genes and antioxidative enzymes in myeloproliferative neoplasms (MPN): polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF).
Methods: Using microarray analysis we studied oxidative stress induced gene expression in $CD34^+$ hematopoietic progenitors of MPN patients. An assay for superoxide dismutases (SOD) was based on the ability of SOD to inhibit the autoxidation of epinephrine at alkaline pH. The activity of glutathione reductase (GR) was based on the capacity of GR to catalyze the reduction of oxidized to reduced glutathione using NADPH as a substrate. Glutathione peroxidase activity was assayed following the oxidation of NADPH with t-butyl-hydroperoxide as a substrate. The antioxidative enzymes activities were determined in red blood cells lyzate.
Results: Oxidative stress induced FBJ murine osteosarcoma viral oncogene homolog (FOS) gene expression was highly elevated in ET (3.1 fold) and PV (3.7 fold) comparing to healthy controls. FOS gene expression was higher in JAK2V617F heterozygous PV patients (4.1 fold). Less prominent expression was observed for kelch-like ECH-associated protein 1 (KEAP1) gene in PV (1.6 fold) and PMF (1.8 fold). Regarding ET patients, heme oxygenase 1 (HMOX1) gene was preferentially expressed in JAK2V617F positive ET (2.4 fold), significantly higher than in healthy controls (p<0.05). Also, HMOX1 was significantly more expressed in JAK2V617F homozygous PV patients (2.5 fold), than in healthy controls (p<0.05). In contrary, v-maf musculoaponeurotic fibrosarcoma oncogene homolog F (MAFF) gene was significantly less expressed in JAK2 homozygous PV and PMF than in healthy controls (p<0.01). The levels of superoxide dismutase and glutathione peroxidase were the most abundant in PMF of MPN. The level of glutathione reductase was the highest in PV, not influenced by JAK2V617F mutant allele burden. However in PMF, the level of glutathione reductase was the most increased in JAK2 homozygous PMF and reduced in JAK2 negative patients, in opposite to glutathione peroxidase levels.
Summary and Conclusions: Presented oxidative stress induced gene expression demonstrated JAK2 dependence in MPN. The antioxidative enzymes activities were the most prominent in PMF. So, oxidative stress effects both at gene and enzyme levels revealed a variation specific for certain type of MPN.

PB1720

GENE EXPRESSION AND PROTEIN LEVELS OF MMPS, TIMPS AND SPARC AND THEIR RELATIONSHIP WITH PLASMA MARKERS OF ANGIOGENESIS IN MYELOFIBROSIS AND ESSENTIAL THROMBOCYTHEMIA PATIENTS
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Background: Myelofibrosis (MF) and essential thrombocythemia (ET) are myeloproliferative neoplasms (MPNs) resulting from acquired hematopoietic stem cell mutations. The molecular mechanisms involved in MPNs pathogenesis are not completely elucidated. It is known that angiogenesis plays an important role in hematological neoplasias progression and prognosis. Matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs) and secreted protein acidic and rich in cysteine (SPARC) are involved in tumor neoangiogenesis. In this context, evaluation of genes related with angiogenesis could be useful to better understand MPN pathobiology.
Aims: To investigate the mRNA and protein expressions of MMPs, TIMPs and SPARC and their relationship with plasma markers of angiogenesis (VEGFA and bFGF) in MF and ET patients.
Methods: Forty-three MPNs patients (MF=29 and ET=14) and 36 control were studied. The controls were matched with patients according age and gender. The MPN diagnosis was defined according World Health Organization criteria (2008). The MMP2, MMP9, TIMP1, TIMP2 and SPARC gene expression were evaluated in peripheral leukocytes and VEGFA and bFGF concentration were measured in plasma from MPN patients. mRNA gene expression and proteins levels were measured by real time PCR and Luminex technology, respectively.
Results: High MMP2, MMP9 and TIMP1 mRNA expression were observed just in MF patients compared to controls (P<0.05). High SPARC mRNA levels was detected in MF and ET patients when compared to controls (P<0.05). In MF group, SPARC mRNA expression was inversely associated with MMP9 expression (β=-0.436 ,R^2=0.3672 and P=0.002) and directly associated with TIMP1 and TIMP2 expression (β=0.625, R^2= 0.4984 and P<0.001; β=0.346, R^2=0.2510 and P=0.015). MF patients showed higher levels of VEGFA and bFGF then controls. Two models of multivariate linear regression were performed in MF