Molecular characterization of Philadelphia-negative myeloproliferative neoplasms

Angela Brisci[1], Daniela Pietra[2], Barbara Foglieni[1*], Sabrina Boggi[2], Elisa Rumi[2], Francesco Passamonti[2], Maurizio Ferrari[1,3,4], Laura Cremonesi[1]

[1]Genomic Unit for the Diagnosis of Human Pathologies, Center for Genomics, Bioinformatics and Biostatistics, San Raffaele Scientific Institute, Milano

[2]Department of Hematology-Oncology, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Pavia

[3]Università Vita-Salute San Raffaele, Milano

[4]Diagnostica e Ricerca San Raffaele SpA, Milano

ABSTRACT

A somatic gain-of-function mutation in exon 14 of the Janus kinase 2 (JAK2) gene is found in Philadelphia-negative myeloproliferative neoplasms, which include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Hematopoietic cells can be either heterozygous or homozygous for JAK2 (V617F) mutation, producing variable proportions of mutant alleles and a relation was observed between mutation status and clinical phenotype. Moreover, while the vast majority of patients with PV carry the V617F mutation in JAK2 exon 14, mutations in exon 12 have been recently reported in V617F-negative patients with PV. For the evaluation of granulocyte JAK2 (V617F) mutation status, we developed both a quantitative real-time polymerase chain reaction (PCR)-based and a microelectronic chip assay as a model of microarray-based approach. A significant correlation was found between the percentages of JAK2 (V617F) mutant alleles estimated by the two methods. However, the real-time PCR approach proved to be more sensitive in the detection of the minority mutant allele than the microelectronic chip. To further characterize JAK2 exon 12 mutations, we performed analysis in 128 patients with JAK2 (V617F)-negative myeloproliferative neoplasms. Direct sequencing analysis of JAK2 exon 12 led to the identification of five different mutations in cases where the levels of the mutated alleles were higher than 10%.

INTRODUCTION

The identification of a somatic gain-of-function mutation in exon 14 of the Janus kinase 2 (JAK2) gene in patients with Philadelphia-negative myeloproliferative neoplasms (Ph-negative MPN) has considerably improved our knowledge of the pathophysiology of these conditions (1-4). The mutation initially occurs in a multipotent hematopoietic stem cell and causes a selective expansion of its myeloid-lineage cell progeny, giving rise to a clone heterozygous for the mutation. As a second step, in a subset of patients a mitotic recombination occurs in a heterozygous hematopoietic cell generating a new homozygous clone that expands and progressively replaces the previous heterozygous clone (3).

The initial data on the main mutation JAK2 (V617F) identified so far were mainly generated by DNA sequencing, which still remains the “gold standard” for molecular characterization of known and new sequence variants despite its low sensitivity (about 10-20% of point mutations in a wild-type background) (1). More sensitive methods have been subsequently employed, resulting that the JAK2 (V617F) mutation can be detected in the vast majority of patients with polycythemia vera (PV), and in over half of those with essential thrombocythemia (ET) and primary myelofibrosis (PMF) (1, 5, 6).

Observations also suggest that both the proportion of mutant alleles and the mutation status of individual cells (heterozygous vs. homozygous state) might contribute in determining the clinical phenotype, as well as provide relevant prognostic information (6, 7). Thus, the aim of molecular diagnosis should be not only the detection of somatic mutations, but rather evaluation of the percentage of chromosomes carrying the specific mutation in a given cell population.

More recently, Scott et al. identified mutations of JAK2 exon 12 in JAK2 (V617F)-negative patients (8) and other studies have confirmed their findings (9-14). These mutations can activate pathways associated with erythropoietin signaling and one of these variants resulted in a PV phenotype in a mouse transplant model (8).

In this work, we developed a quantitative real-time polymerase chain reaction (qRT-PCR)-based allelic discrimination assay to evaluate the granulocyte JAK2 (V617F) mutation status. In parallel, in the search of alternative sensitive diagnostic strategies, we also evaluated the microelectronic chip technology as a model of microarray-based approach. In the past, we successfully applied this system to the identification of several disease-gene mutations and, in particular, to the detection of minority fetal mutated alleles in maternal plasma for non-invasive prenatal diagnosis (15-17). Finally, for a comple-

*Present address: Department of Transfusion Medicine and Hematology, Ospedale Alessandro Manzoni, Lecco.

te molecular characterization of sequence variations in exon 12 of the JAK2 gene, samples from patients with JAK2 (V617F)-negative MPN were also subjected to direct sequencing.

MATERIALS AND METHODS

Patients

According to the 2008 WHO and the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) diagnostic criteria (18), 451 subjects were evaluated for JAK2 (V617F) mutation by microelectronic chip and qRT-PCR: 112 patients with PV, 165 with ET, 41 with PMF, 17 with post PV myelofibrosis (post-PV MF), 11 with post ET myelofibrosis (post-ET MF), 8 familial PV, 33 idiopathic erythrocytosis (IE), and 64 secondary cytosys (SC). Moreover, 128 patients with MPN (30 sporadic PV, 26 familial PV, and 72 IE) were analysed for JAK2 exon 12 mutations. All patients were followed at the Department of Hematology-Oncology, Fondazione IRCCS Policlinico San Matteo and University of Pavia. All the investigations were approved by the local ethic committee; the procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000, and samples were obtained after subject written informed consent.

Cells separation and DNA isolation

Peripheral blood granulocytes were obtained from the neutrophil fraction by osmotic lysis of red cells after Ficoll gradient centrifugation.

DNA extraction was performed using the Puregene Blood DNA isolation kit (Qiagen).

Microelectronic chip

PCR conditions

For the detection of JAK2 (V617F) mutation primers were designed to amplify a region of 188 bp encompassing exon 14 (primers: forward 5'-CCTTAGTCTTTCTTTGAAGCAGCA-3', reverse biotin-5'-ACTGACACCTAGCTGTGATCC-3').

PCR was performed in 50 μL reactions containing 100 ng DNA, 200 μM deoxyribonucleotide triphosphates (dNTPs) each, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1.3 U of thermostable DNA polymerase (AmpliTaq Gold Applied Biosystem) and 20 pM of each primer. Cycling conditions were 95 °C for 10 min (one cycle); 95 °C for 30 s, 60 °C for 30 s, 72 °C for 30 s, for a total of 35 cycles, and 72 °C for 7 min (one cycle).

PCR products purification and desalting was performed with the Multiscreen separation system (Millipore Corporations) by 96-well plates (Multiscreen-PCR Plates MANU 030). 30 μL of each sample were then mixed with histidine to a final 50 mM concentration and addressed to the chip.

Instrumentation

We employed the NMW 1000 NanoChip™ molecular biology workstation by Nanogen, Inc. In this system, the biotinylated amplicon is electronically addressed to specific positively charged test sites on the cartridge, where it remains embedded through interaction with streptavidin in the permeation layer covering the microelectronic chip. Details of chip microfabrication, instrumentation, application of the permeation layer and protocols for sample loading and deposition were previously described (15, 16, 19-21).

Reporter and stabilizer design

The two reporters, specific for either the wild-type or the mutant sequence, labeled with fluorescent tags and stabilizer oligonucleotides to open the secondary structure of the template DNA were designed with the help of web-free programs (DNAmfold: http://www.bioinfo.rpi.edu/Ezukerm/rna and OligoAnalyzer 3.0: http://www.idtdna.com). A universal labeling format based on reporters containing a discriminator tail complementary to either a wild-type or a mutant universal 3' Alexa532/Alexa647 labeled probe was used (22). Reporters were designed to have the base variation located in the middle according to a dot-blot format, as follows: wild-type reporter 5'-ctgagtccgaacattgagGTATGTGTCTGTG-3', mutant reporter 5'-gcagtatatcgcttgacaAGTATGTTTCTGTG-3' (small letters: discriminator tail; capital letters: reporter, with the variant base underlined); wt universal probe ctcaatgttcggactcag-3'-Alexa532, mutant universal probe tgtcaagcgatatactgc-3'-Alexa647. Moreover, a stabilizer oligonucleotide was necessary: 5'-AGACGAGAGTAAGTAAAACTACAGGCTTTCTAATGC-3'.

Hybridization, thermal stringency and fluorescence detection

After electronic addressing, PCR products were denatured and incubated with the specific stabilizer and reporter probes; following hybridization, a thermal stringency step at 32 °C was performed (15, 16, 19).

Hybridization was detected by fluorescence automated scanning through a dedicated software. Only samples displaying a signal-to-noise ratio greater than 5-fold were considered.

An artificial heterozygous control sample for V617F mutation, containing G1849T nucleotide substitutions, was used as a reference standard to evaluate the ratio of the wild-type and mutant alleles in the unknown samples analyzed (23).

Quantitative real-time PCR (qRT-PCR)

A qRT-PCR-based allelic discrimination assay was developed for the identification of the JAK2 (V617F) mutation (6). A single common forward primer and two reverse primers were designed based on the nucleotide difference at the 3' terminal base for allelic discrimination of the G to T transversion. In order to prevent the amplification of the non matching primer, an additional nucleotide mismatch (G to T) located three bases from the 3' termini of the allele-specific primers was incorporated.

PCR results were analyzed applying the cycle threshold (ΔCt) method and for each sample the percentage of JAK2 (V617F) alleles was calculated.

Sequencing

For JAK2 exon 12 mutation, screening primers were designed to amplify a region of 453 bp containing the whole exon 12 (primers: forward 5'-CAAAGTTCAATGAGTTGACCCCTA-3', reverse 5'-ACACAAGGTTGGCATATTTTTCATAAG-3'). PCR was performed in 50 μL reactions containing 300 ng DNA, 200 μM dNTPs each, 1.5 mM $MgCl_2$, 1.5 U of Dynazyme DNA polymerase together with 1X Mg-free buffer (Finnzymes) and 15 pM of each primer. Cycling conditions were 95 °C for 10 min (one cycle); 95 °C for 30 s, 55 °C for 20 s, 72 °C for 20 s (35 cycles), and 72 °C for 7 min (one cycle). Sequencing was performed using the Big Dye Terminator v1.1 Cycle Sequencing kit and an ABI Prism 3130XL genetic analyzer (Applied Biosystems).

RESULTS

Microelectronic chip analysis and qRT-PCR allelic discrimination assay

Optimized conditions for the correct identification of either mutant or wild-type alleles for the JAK (V617F) mutation were set up by the microelectronic chip. An artificial heterozygous control sample was used as a reference standard to evaluate the mutant: wild-type (M:W) ratios in patients with unknown genotype.

At the beginning, we evaluated the reproducibility of the assay by amplifying the first 20 samples in double and addressing each amplified product onto two different microelectrodes. The variability (CV%) of the M:W allele ratio for two amplified products from the same sample was 17.6%, calculated on the mean value obtained from two microelectrodes; the variability of the M:W ratio between the two microelectrodes analyzed for the same sample was 12.8%. Due to the high reproducibility of the system, we then analyzed all the remaining 431 samples by singularly amplifying each sample and addressing it to a single microelectrode.

Figure 1 shows examples of samples displaying different M:W ratios analyzed by the microelectronic chip approach. The occurrence of the mutation was found to be approximately 95% in PV and 50% in both ET and PMF, with a 5% threshold detection limit of the minority mutant alleles. Less than 5% mutated allele was considered background signal.

These results significantly correlated with those obtained on the same granulocyte samples by qRT-PCR allelic discrimination assay (r=0.967) (Table 1). This last method was more sensitive, allowing the identification of 1% of the minority mutant alleles in the samples analyzed. In fact, dilution experiments showed that the lower detection limit was 0.5% of mutant alleles. However, to avoid false positive results, a cutoff of 1% of mutant alleles was chosen to define a patient as positive.

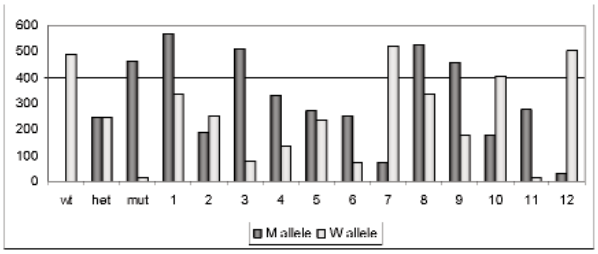
Mutational screening of JAK2 exon 12

JAK2 exon 12 mutations were detected in 10 out of 128 patients (7.8%) with confirmed MPN. We identified 5 different mutations, which include 3 deletions of 6 nucleotides and two previously unreported duplications that modified the rest of the sequence by adding 33 bp: R541-E543delinsK, N542-E543del, E543-D544del, V536-I546dup11, F537-I546dup10 + F547L (14).

All mutations were detected in peripheral blood granulocytes, but not in T lymphocytes of the patients, thus demonstrating the somatic nature of the variants. The pattern on sequencing was heterozygous in all patients but one. An example is shown in Figure 2.

DISCUSSION

Detection of the JAK2 (V617F) mutation currently represents an extremely useful diagnostic tool in the management of Ph-negative MPN. Using sensitive assays, the JAK2 (V617F) mutation can be observed in approximately 95% of patients with PV and about half of those with ET or PMF, and its detection is now a major criterion for the diagnosis of MPN (18). Moreover, evaluation of granulocyte mutant alleles has been shown to



Sample	M allele	W allele	M:W Ratio	% M allele
wt	1	491	1:491	0.2
het	246	246	1:1	50.0
mut	461	14	31.89:1	97.0
1	567	335	1.7:1	62.9
2	186	249	1:1.4	42.8
3	507	77	6.6:1	86.8
4	330	135	2.4:1	71.0
5	271	235	1.1:1	53.6
6	254	75	3.4:1	77.2
7	72	520	1:7.2	12.2
8	527	337	1.6:1	61.0
9	458	180	2.5:1	71.8
10	176	402	1:2.3	30.4
11	275	16	17.2:1	94.5
12	28	503	1:18	5.3

Figure 1
Example of a microelectronic chip analysis as recorded by the Nanogen NanoChip molecular workstation. Analysis of the V617F (G1849T) mutation at 32 °C thermal stringency. Fluorescence signals after hybridization for wild-type (W) (grey) and mutant (M) (black) reporters for samples displaying different M:W ratios are shown. Samples are reported on the x-axis as wt (wild-type control), het (heterozygous control), mut (homozygous mutated control) and 1-12 (patients).
In the table, mutant and wild-type allele fluorescence signals (M and W allele), allele ratio (M:W), and percentage of the minority mutant allele (% M allele) are listed for each sample. Only samples displaying a signal-to-noise ratio greater than 5-fold are considered.

Table 1
Frequency of JAK2 (V617F) mutation found by microlectronic chip and quantitative real-time polymerase chain reaction (qRT-PCR) on peripheral blood granulocytes in subjects with different pathological conditions

Disease	n	V617F-positive (%)	
		Microelectronic chip	qRT-PCR
PV	112	104 (92.9)	105 (93.8)
ET	165	92 (55.8)	96 (58.8)
PMF	41	24 (58.5)	23 (56.1)
Post-PV MF	17	17 (100.0)	17 (100.0)
Post-ET MF	11	6 (54.5)	6 (54.5)
Familial PV	8	4 (50.0)	4 (50.0)
IE	33	0	0
SC	64	0	0

PV, polycythemia vera; ET, essential thrombocythemia; PMF, chronic idiopathic myelofibrosis; post-PV MF, post-polycythemia vera myelofibrosis; post-ET MF, post-essential thrombocythemia myelofibrosis; IE, idiopathic erythrocytosis; SC, secondary cytosys.

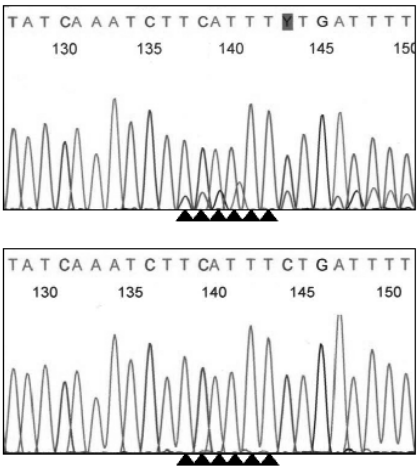


Figure 2
Representative granulocyte and T lymphocytes DNA sequence chromatograms (antisense strand) from a patient with N542-E543del in exon 12 of the JAK2 gene. Traces reveal the somatic mutation indicated by arrowheads.

ntribute in determining the clinical phenotype, as well as to provide relevant prognostic information. In fact, PV patients have higher proportions of granulocyte mutant alleles (5, 6, 24) and of homozygous progenitor cells (7) than ET ones. A mutant allele dosage effect on erythrocytosis and leukocytosis has been observed in PV (25) and patients with high mutant allele burden have been found to have a more severe disease (26).

As a consequence, new challenges occur to a diagnostic laboratory in order to design assays for both detecting and evaluating granulocyte JAK2 (V617F) mutation status. Sequencing and allele-specific-PCR (AS-PCR) have been employed to detect JAK2 mutations, each with its particular positive and negative peculiarities (1). Even if sequencing is commonly considered the "gold standard" method for mutation scanning because it allows the characterisation of novel mutations, it is unlikely to detect percentages of mutant alleles lower than 10%, corresponding to a threshold of 20% granulocytes heterozygous for the mutation. On the other hand, AS-PCR is highly sensitive, but it is restricted to known mutations and requires multiple reactions being performed to cover the whole mutations spectrum. At any rate, neither of them provide a sort of percentage of mutant alleles. In consideration of the approximately 5% of JAK2 (V617F)-negative PV cases, the recent description of mutations in exon 12 of the JAK2 gene contribute to genetic background of this disorder (8). Thus, it is also important to study and identify new acquired lesions in JAK2 gene.

In this work, we developed a qRT-PCR-based allelic discrimination assay and a reliable and sensitive microelectronic chip approach for the evaluation of the granulocyte JAK2 (V617F) mutation status. We estimated the percentages of JAK2 (V617F) alleles in 451 subjects using both methods obtaining a full concordance of results.

Our study confirm that the microarray assay may be applied to rapid, large scale population screening for mutations thanks to the simplicity of the chip analysis and its possibility to analyze a relatively elevated number of patients in a single test for several loci. The microelectronic chip is characterized by a 5% threshold detection limit of the minority mutant alleles that is higher than the sensitivity value reported by Di Ianni et al. when using a similar approach (27). This discrepancy may be due both to a different primers, stabilizers and reporters design and to the use of different mutated control samples for sensitive assays, i.e. artificial homozygous PCR product in our study and triploid HEL cells in the previous one. The microarray assay used in this work may represent the first step towards the development of new platforms based on the use of highly innovative substrates for high-sensitive genotyping of minority mutated alleles allowing to identify very low percentages (about 1%) (28).

In parallel, direct sequencing to characterize known and new variants led to the detection of 8 mutations, including two previously unreported ones, but was not able to identify levels of mutant alleles lower than 10% (14).

From all these results it becomes evident that in the molecular analysis of pathologies characterized by the presence of variable amounts of minority mutant alleles, the diagnostic strategy cannot rely on a unique methodological approach. Due to the heterogeneity of causative mutations, the use of a sequencing method to characterize known and new sequence variants is unavoidable. On the other hand, due to their low sensitivity, current sequencing methods need to be coupled with high sensitive technologies to scan for new variations, detect known mutations at the lowest proportion and, ideally, provide a percentage of mutant alleles.

In conclusion, JAK2 mutations in patients with Ph-negative MPN have great significance in the understan-

ding of the molecular mechanisms underlined the pathogenesis of these diseases. Evaluation of the granulocyte mutation status by sensitive approaches and the identification of new acquired lesions involved in the same JAK-Signal Transducer and Activator of Transcription (STAT) pathway activation provides new specific diagnostic, prognostic and therapeutic tools, as the development of novel anti-JAK2 small molecule drugs, contributing to clinical management of patients (29, 30).

ACKNOWLEDGMENTS

This study was supported by grants from Associazione Italiana per la Ricerca sul Cancro (AIRC), Milano, Fondazione Cariplo, Milano, Fondazione Ferrata Storti, Pavia, and Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, Pavia, Italy.

REFERENCES

1. Baxter EJ, Scott LM, Campbell PJ, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet 2005;365:1054-61.
2. James C, Ugo V, Le Couedic JP, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature 2005;434:1144-8.
3. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med 2005;352:1779-90.
4. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell 2005;7:387-97.
5. Lippert E, Boissinot M, Kralovics R, et al. The JAK2-V617F mutation is frequently present at diagnosis in patients with essential thrombocythemia and polycythemia vera. Blood 2006;108:1865-7.
6. Passamonti F, Rumi E, Pietra D, et al. Relation between JAK2 (V617F) mutation status, granulocyte activation, and constitutive mobilization of CD34+ cells into peripheral blood in myeloproliferative disorders. Blood 2006;107:3676-82.
7. Scott LM, Scott MA, Campbell PJ, et al. Progenitors homozygous for the V617F mutation occur in most patients with polycythemia vera, but not essential thrombocythemia. Blood 2006;108:2435-7.
8. Scott LM, Tong W, Levine RL, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med 2007;356:459-68.
9. Pardanani A, Lasho TL, Finke C, et al. Prevalence and clinicopathologic correlates of JAK2 exon 12 mutations in JAK2V617F-negative polycythemia vera. Leukemia 2007;21:1960-3.
10. Percy MJ, Scott LM, Erber WN, et al. The frequency of JAK2 exon 12 mutations in idiopathic erythrocytosis patients with low serum erythropoietin levels. Haematologica 2007;92:1607-14.
11. Martinez-Aviles L, Besses C, Alvarez-Larran A, et al. JAK2 exon 12 mutations in polycythemia vera or idiopathic erythrocytosis. Haematologica 2007;92:1717-8.
12. Butcher CM, Hahn U, To LB, et al. Two novel JAK2 exon 12 mutations in JAK2V617F-negative polycythaemia vera patients. Leukemia 2008;22:870-3.
13. Williams DM, Kim AH, Rogers O, et al. Phenotypic variations and new mutations in JAK2 V617F-negative polycythemia vera, erythrocytosis, and idiopathic myelofibrosis. Exp Hematol 2007;35:1641-6.
14. Pietra D, Li S, Brisci A, et al. Somatic mutations of JAK2 exon 12 in patients with JAK2 (V617F)-negative myeloproliferative disorders. Blood 2008;111:1686-9.
15. Foglieni B, Cremonesi L, Travi M, et al. Beta-thalassemia microelectronic chip: a fast and accurate method for mutation detection. Clin Chem 2004;50:73-9.
16. Santacroce R, Ratti A, Caroli F, et al. Analysis of clinically relevant single-nucleotide polymorphisms by use of microelectronic array technology. Clin Chem 2002;48:2124-30.
17. Galbiati S, Foglieni B, Travi M, et al. Peptide-nucleic acid-mediated enriched polymerase chain reaction as a key point for non-invasive prenatal diagnosis of beta-thalassemia. Haematologica 2008;93:610-4.
18. Barosi G, Mesa RA, Thiele J, et al. Proposed criteria for the diagnosis of post-polycythemia vera and post-essential thrombocythemia myelofibrosis: a consensus statement from the International Working Group for Myelofibrosis Research and Treatment. Leukemia 2008;22:437-8.
19. Ferrari M, Cremonesi L, Bonini P, et al. Single nucleotide polymorphism and mutation identification by the Nanogen microelectronic chip technology. In: Joos T, Fortina P, eds. Methods in molecular medicine. Microarray in clinical diagnostics. Totowa, NJ: Humana Press Inc, 2005;144:93-106.
20. Gilles PN, Wu DJ, Foster CB, et al. Single nucleotide polymorphic discrimination by an electronic dot blot assay on semiconductor microchips. Nat Biotechnol 1999;17:365-70.
21. Sosnowski RG, Tu E, Butler WF, et al. Rapid determination of single base mismatch mutations in DNA hybrids by direct electric field control. Proc Natl Acad Sci U S A 1997;94:1119-23.
22. Cooper KL, Goering RV. Development of a universal probe for electronic microarray and its application in characterization of the Staphylococcus aureus polC gene. J Mol Diagn 2003;5:28-33.
23. Stenirri S, Fermo I, Battistella S, et al. Denaturing HPLC profiling of the ABCA4 gene for reliable detection of allelic variations. Clin Chem 2004;50:1336-43.
24. Moliterno AR, Williams DM, Rogers O, et al. Molecular mimicry in the chronic myeloproliferative disorders: reciprocity between quantitative JAK2 V617F and Mpl expression. Blood 2006;108:3913-5.
25. Tefferi A, Lasho TL, Schwager SM, et al. The clinical phenotype of wild-type, heterozygous, and homozygous JAK2V617F in polycythemia vera. Cancer 2006;106:631-5.
26. Vannucchi AM, Antonioli E, Guglielmelli P, et al. Clinical profile of homozygous JAK2 617V>F mutation in patients with polycythemia vera or essential thrombocythemia. Blood 2007;110:840-6.
27. Di Ianni M, Moretti L, Del Papa B, et al. A microelectronic DNA chip detects the V617F JAK-2 mutation in myeloproliferative disorders. Leukemia 2006;20:1895-7.
28. Galbiati S, Damin F, Di Carlo G, et al. Development of new substrates for high-sensitive genotyping of minority mutated alleles. Electrophoresis 2008;29:4714-22.
29. Tefferi A. Essential thrombocythemia, polycythemia vera, and myelofibrosis: current management and the prospect of targeted therapy. Am J Hematol 2008;83:491-7.
30. Pardanani A. JAK2 inhibitor therapy in myeloproliferative disorders: rationale, preclinical studies and ongoing clinical trials. Leukemia 2008;22:23-30.