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BIOMEDICINE

Detection of *V617F* mutation in gene *jak2* at patients with chronic myeloproliferative neoplasms

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The aim *of the work was to create a protocol for detecting the V617F mutation of the gene jak2 in samples of patients with chronic myeloproliferative neoplasm which is necessary to unify the procedures of the analysis of blood samples according to WHO criteria for this group of diseases.* ***Methods****. Mutation was revealed using reverse transcriptase PCR and direct sequencing of PCR products.* ***Results****. Six samples of blood of patients with polycythemia vera were analyzed and the mutation V617F was detected in all six cases. This mutation was not detected in any of RNA samples of healthy donors. A case of simultaneous detection of mutations V617F and fused bcr/abl gene in CML patient was described.* ***Conclusions****. The proposed method for detecting the V617F mutation allows molecular genetic differential diagnosis of myeloproliferative neoplasm as well.*

Keywords: Jak2, V617 F, myeloproliferative neoplasms.

Introduction Pursuant to 2008 edition of WHO classification it was suggested to change the term "chronic myeloproliferative diseases" for a new one – "myeloproliferative neoplasms (MNP)". Myeloproliferative neoplasms are divided into two groups – classic ones (chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocytosis (ET), idiopathic myelofibrosis (IMF)) and neoclassic ones (atypical CML, chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia / hypereosinophilic syndrome, non-classified CML) [1].

Besides changing the name of this group of pathologies, noteworthy is an improvement of diagnostic criteria, especially a wider application of molecular and genetic markers.

It is known that among myeloproliferative diseases only CML has clearly described cytogenetic marker – Philadelphia chromosome, and gene *bcr/abl* at the molecular level. However, in 2005 several groups of researchers described [2-6] a point mutation G>T in the exon 14 of gene *jak2*, which causes the replacement of valine by phenylalanine in the position 617 (*V617F*). This mutation is revealed in 90–95% of patients with PV and approximately in 50% of patients with ET and IMF. It is believed that at the functional level this replacement causes a loss of regulation and constitutive activation of JAK2-tyrosine kinase. Subsequently, it results in the loss of regulation of the JAK-STAT-signaling pathway and corresponding increase in the cell transcriptional activity and in the number of erythrocytes, thrombocytes and granulocytes.

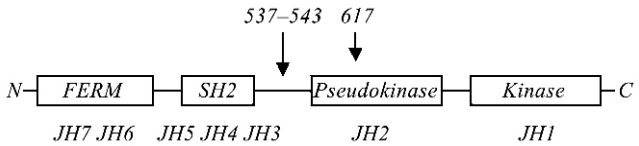

Fig.1. The structure of protein JAK2 and localization of mutations . Domains: *JH6–JH7 – FERM* homological (*F* – with protein 4.1, *E* – ezrin, *R* – radixin, *M* – moesin); *JH3–JH5 – SH2* (*SRC2* homological); *JH2* – Pseudokinase (pseudokinase); *JH1* – Kinase (tyrosinekinase). Localization of mutations marked with arrows (aminoacids position)

Besides the *V617F* mutation, a series of changes were described in the exon 12 of gene *jak2* (K539L, H538QK539L, F537–K539delinsL, etc.) [7] – predominantly in the patients with PV (up to 5%). The structure of protein JAK2 and localization of mutations are presented in Fig. 1.

However, the abovementioned evidences to the fact that though the *V617F* mutation is not specific for one particular disease it can play an important role in diagnostics. For instance, it is absent at secondary polycythaemia, reactive thrombocytosis or secondary bone marrow fibrosis. The absence of this mutation in the patients with polycythaemia requires the search for some other causes of erythrocytosis.

As it was stated above, this mutation is revealed in 50% of the patients with ET and IMF which is also a powerful diagnostic criterion. However, it should be noted that revealing the mutation is an important but not self-sufficient criterion for diagnostics of myeloproliferative neoplasms. Therefore, the diagnostics may be considered reliable only in the complex with clinical and laboratory methods, first of all, the analysis of bone marrow cells to exclude fibroses, dysplasia, the presence of blast cells and additional chromosomal anomalies. The proposals for improving the diagnostic criteria for this group of diseases are published in [8–10].

Materials and Methods Blood samples of patients, who were treated in Kiev hematological clinics (with their informed consent), were used in the work. RNA was obtained by the method described in [11]. The reaction mixture for cDNA synthesis contained 1–3 μg RNA, buffer 1 for reverse transcriptase, 0.1 μg of primer Random Hexamer Primer (*Fermentas*, Lithuania), 300 units of reverse transcriptase RevertAid™ M-MuLV Reverse Transcriptase (*Fermentas*), 20 units of RNasine (*Promega*, USA), 1 mM dNTP. The reaction was performed at 42°C for 1 h and stopped by heating (70°C, 10 min), 2 μl of reaction mixture were used for amplification. PCR was performed using specific primers which were selected by Generun software:

J1F – 5'-CACCAACATTACAGAGGCCTAC-3';

J1R – 5'-GCCAGGATCACTAAGTTTGATG-3' (the length of amplificate – 536 b. p.);

J2F – 5'-CGGTCAACTGCATGAAACAG-3',

J2R – 5'-TTGGCACATACATTCCCATG-3' (the length of amplificate – 321 b.p.).

PCR was performed in the volume of 30μl for 30 cycles (94°C – 35 s; 55°C – 35 s; 72°C – 45 s), using 10 picomol of primers J1F and J1R.

The primers to reveal -actin (5'-GCTCGTCGTC GACAACGGCTC-3' and 5'-CAAACATGATCTGG GTCATCTTCTC-3') (*Invitrogen*, USA) and/or 18S rRNA (5'-CGGCTACCACATCCA AGGAA-3' and 5'-GCTGGAATTACCGCGGCT-3') were used to control the synthesis of cDNA. [The] Amplification products were analyzed in 2% agarose gel.

The second stage of PCR was conducted, using 0.5 μl of obtained amplificate and 10 picomol of primers J2F and J2R, to confirm specificity and in case of insufficient amount of amplificate for sequencing.

The PCR products obtained were purified using QIAquick PCR Purification Kit (*Qiagen*, USA) or extraction with chloroform and precipitation with ethanol; then they were sequenced using primers J2F and J2R. The obtained sequences were analyzed using BioEdit and BLAST software.

Results and Discussion The *V617F* mutation in gene *jak2* was revealed using reverse transcriptase PCR and direct sequencing of PCR products in the patients with myeloproliferative neoplasms. It is possible to reveal this mutation without reverse transcriptase, but according to diagnostic criteria of WHO, it is necessary to determine the presence of fused gene *bcr/abl* in such patients. Due to specificities of the structure of abovementioned gene, the RT-PCR is required for this purpose, therefore, we consider it necessary not to isolate separately RNA and DNA, but to combine the procedures of RNA and cDNA isolation to reveal both themutations in gene *jak2* and fused gene *bcr/abl*. Besides, a series of mutations in the positions

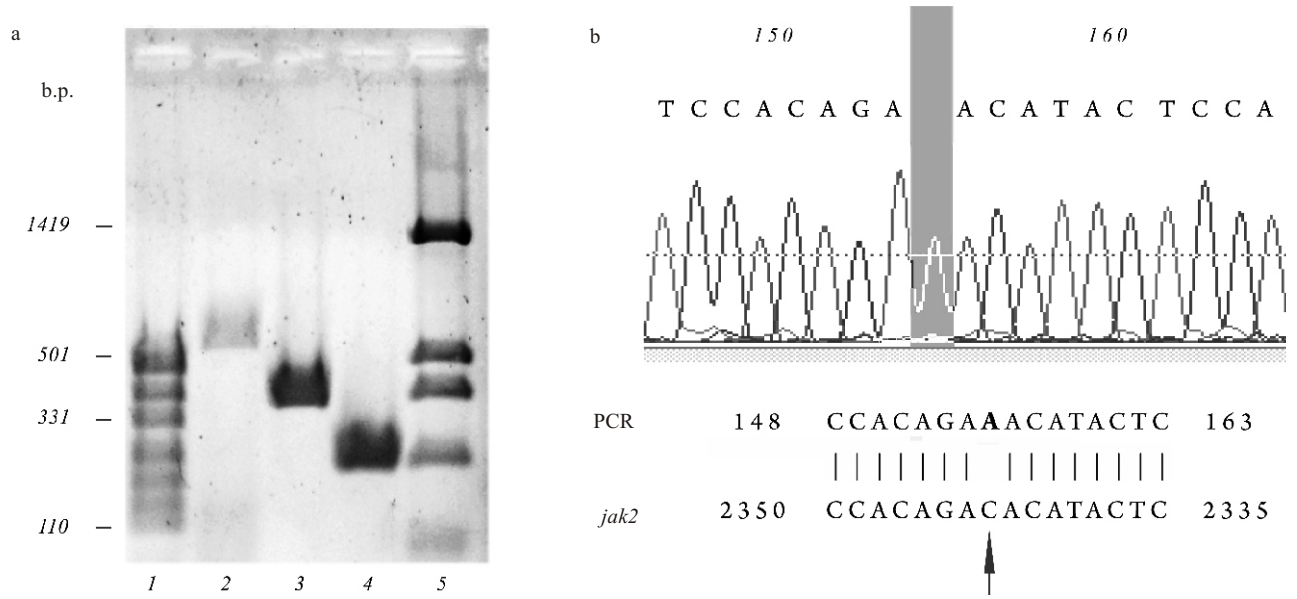

Fig. 2. The electrophoregram (a) and a fragment of the PCR product, obtained while examining patient B blood (b).(*a*: *1* – molecular mass marker *pUC19/MspI*; *2* – PCR product, obtained from patient B blood; *3*, *4* – positive control of RT-PCR (-actin and 18S rRNA, respectivly); *5* – molecular mass marker *pUC19/HinfI*; *b*:replacement corresponding to *V617F* mutation marked with inversion. (Bases of *jak2 gene* numbered according to sequence of NM_004972.2 (*Homo sapiens* Janus kinase 2))

607, 611, 616, and 619 of JAK2 also disturb the functioning of gene *jak2*, and sequencing permits to reveal these variants as well.

Patient B, born in 1950, suffered from polycythaemia vera since 1998. Fig.2 presents the electrophoregram and a fragment of the PCR product, obtained while examining his blood. As seen, the search for homology of sequence of the PCR product with the mRNA *jak2* (NM_004972.2 *Homo sapiens* Janus kinase 2) using BLAST software revealed the replacement C>A in position 2343. At the protein level it causes replacement of valine by phenylalanine in the position 617, i.e. *V617F* mutation.

In total, six blood samples of patients with PV were analyzed and the *V617F* mutation was detected in all six cases (in three of them this mutation was heterozygous). The abovementioned mutation was not detected in any of five control samples of healthy donors.

At present, only several cases of the *V617F* mutation are described in patients with CML. However, the *V617F* mutation was also detected in the blood sample of patient G, born in 1938, the presence of Philadelphia chromosome in which was proven by detection of the fused gene *bcr/abl* using RT-PCR in accordance to the methodological recommendations [12].

As it has been stated above, only several cases of mutation in gene *jak2* are known, thus, the nature of this phenomenon is yet to de defined. Two main explanations are now under consideration: the mutations are simultaneously revealed due to the presence of two different clones, each of which carries one mutation [13, 14], or both mutations are localized in the same clone, and the mutation in gene *jak2* precedes formation of the chimeric gene *bcr/abl* [15, 16]. However, both assumptions require further research.

The data presented evidence to efficiency of the suggested protocol for the detection of *V617F* mutation by RT-PCR and direct sequencing, which allows using it for molecular and genetic differential diagnostics of myeloproliferative neoplasms.

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Виявлення мутації *V617F* гена *jak2* у хворих на хронічні мієлопроліферативні неоплазми

Резюме

Мета. *Створити протокол, який дозволяє виявляти мутацію V617F гена jak2 у зразках РНК хворих на хронічні мієлопроліферативні неоплазми, що неохідно для уніфікації процедур аналізу зразків крові згідно з чинними критеріями ВОЗ для даної групи захворювань.* ***Методи.*** *Мутацію визначали за допомогою зворотно-транскриптазної полімеразної ланцюгової реакції та прямого секвенування продуктів полімеразної ланцюгової реакції.* ***Результати.*** *Проаналізовано шість зразків крові хворих на справжню поліцитемію і у всіх випадках виявлено мутацію V617F. Дану мутацію не знайдено в жодному з контрольних зразків РНК здорових донорів. Описано випадок одночасного виявлення мутації V617F та злитого гена bcr/abl у хворої на хронічну мієлоїдну лейкемію.* ***Висновки***. *Запропонований метод дозволяє визначати мутацію V617F, що дає змогу використовувати його для мо- лекулярно-генетичної диференційної діагностики мієлопроліферативних неоплазм.*

Ключові слова: jak2, V617 F, мієлопроліферативні неоплазми.

М. В. Дыбков, И. Р. Гартовская, С. С. Малюта, Г. Д. Телегеев

Выявление мутации *V617F* гена *jak2* у больных
с хроническими миелопролиферативными неоплазмами

Резюме

Цель. *Создание протокола для виявления мутации V617F гена jak2 в образцах РНК больных с хроническими миелопролиферативными неоплазмами, что необходимо для унификации процедур анализа образцов крови согласно настоящим критериям ВОЗ для данной группы заболеваний.* ***Методы.*** *Мутацию определяли с помощью обратно-транскриптазной полимеразной цепной реакции и прямого секвенирования продуктов ПЦР.* ***Результаты.*** *Проанализированы шесть образцов крови больных истинной полицитемией и во всех случаях обнаружена мутация V617F. Эта мутация не найдена ни в одном из контрольных образцов РНК здоровых доноров. Описан случай одновременного выявления мутации V617F и слитого гена bcr/abl у больной с хронической миелоидной лейкемией.* ***Выводы.*** *Предложенный метод позволяет определять мутацию V617F, его также можно использовать для молекулярно-генетической дифференциальной диагностики миелопролиферативных нео- плазм.*

Ключевые слова: jak2, V617 F, миелопролиферативные неоплазмы.

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