

SHORT COMMUNICATIONS

DELETION IN *dbl* DOMAIN OF *bcr/abl* GENE IN LEUKEMIA PATIENTS WITH Ph' CHROMOSOME

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ДЕЛЕЦИИ В УЧАСТКЕ *dbl* ГЕНА *bcr/abl* У БОЛЬНЫХ ЛЕЙКОЗОМ С НАЛИЧИЕМ Ph'-ХРОМОСОМЫ

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In some leukemia patients with Ph' chromosome the presence of deletions in the *dbl* domain of hybrid *bcr/abl* gene was shown. The possible influence of such genetic alterations on peculiarities of disease course is discussed.

Key Words: deletion, *bcr/abl* gene, *dbl* domain, Ph' chromosome, leukaemia.

У некоторых больных лейкемией с наличием Ph'-хромосомы обнаружены делеции в *dbl* домене гибридного гена *bcr/abl*. Обсуждается возможное влияние подобных изменений на особенности течения заболевания.
Ключевые слова: делеции, ген *bcr/abl*, домен *dbl*, Ph' хромосома, лейкемия.

Some forms of leukemia are characterized by the presence of Philadelphia (Ph') chromosome. Ph' chromosome (firstly found in patients with chronic myeloid leukemia (CML) in 1960 [1]) is detected in more than 95% of patients with CML, in 30% of adults and 2–10% of pediatric patients with acute lymphoblastic leukemia (ALL), in approximately 2% of cases of acute myeloid leukemia and in some cases of malignant lymphoma and multiple myeloma [2].

Ph' chromosome is formed by reciprocal translocation between chromosomes 9 and 22 (t(9;22) (q34;q11)) [3]. Due to that translocation the hybrid gene *bcr/abl* is formed: 5'-domain of *bcr* gene of chromosome 22 became linked to 3'-domain of *abl* gene of chromosome 9. The breaks in *abl* gene occur in its 5' region with the length 300000 b.p. predominantly in 3 domains: in the intron domain before 1b exon, in the intron between exons 1b and 1a or in the intron between exons 1a and 2. In the majority of cases the breaks in *bcr* gene occur in M-bcr region (major-bcr) (12–16 exons), as well as in m-bcr (minor bcr) (the intron between exons 1 and 2) and μ -bcr (micro bcr) (exons 17–20) [2,4].

Thus, the hybrid *bcr/abl* gene contains exons 2–11 of *abl* gene (COOH-terminus of the hybrid protein BCR/ABL, respectively) and various in the length (due to the break-points) fragments of *bcr* gene (NH₂-terminus of the hybrid protein BCR/ABL). By translation of those genes p190, p210, p230 proteins are produced.

It has been shown that the clinical features of the disease are associated with the length of *bcr/abl* gene product. Really, the protein p230 BCR/ABL is detected in patients with relatively benign chronic neutrophilic leukemia, the protein p190 BCR/ABL — mostly in patients with ALL, and p210 BCR/ABL is detected

both in ALL and CML patients [4]. The presence of different p210 and p190 proteins seems not to influence the course of the disease in adult patients with ALL. From other hand, in the presence of p210 protein different clinical features in CML and ALL are observed.

We have analyzed the domain of the fusion *bcr/abl* gene, which distinguish p210 BCR/ABL protein from p190 protein (namely, exons 3–10, or *Dbl* domain of *bcr* gene). Possibly, the variations in that domain may influence the different outcome of the disease.

In the work the samples of the blood from patients cured in the hematological hospitals of Kyiv were used. RNA was obtained according to [5]. For cDNA synthesis the specific primer A₁ (5'-TGATTATAGCCTAAGAC-CCGGA-3') and reverse transcriptase M-MuLV (Gibco BRL, USA) were used. The reaction was carried out in 40 μ l (1–2 μ g of RNA, 10 pM of A₁ primer, 10–20 U RNAsin, 1mM dNTP, 20 U of reverse transcriptase). The synthesis was carried out at 37 °C during 1 h. For amplification 5 μ l of reaction mixture were used. After detection of Ph'-chromosome in the blood samples (according to [6]) and the estimation of the rearrangement type the samples with p210 *bcr/abl* rearrangement were selected. For further analysis the *dbl* domain was amplified by polymerase chain reaction (PCR). Reaction was performed as two-round PCR. On the first stage the primers used for amplification were: ext1 *dbl* (5'-GGCTGCCCTACATTGAT-GACTCGC-3') and extr1 *dbl* (5'-GATGTTGGGCACT-GCCTCCAGTTC-3'). The amplification mixture contained 200 μ M dNTP, 10 pM of primers, 5 U of Taq polymerase, an aliquote of freshly synthesized cDNA, and consisted from 30 cycles (94 °C – 30 s, 55 °C – 30 s, 72 °C – 1.5 min). On the second stage 1 μ l of reaction mixture from 1-st stage PCR was used. The amplification was carried out using internal primers: extdbl (5'-AAGCTTGCCCTGGAGTCCACTAAAG-3') and extr *dbl* (5'-GAATTCTGCCTCCAGTTCATCCAC-

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Abbreviations used: ALL — acute lymphoblastic leukemia; CML — chronic myeloid leukemia; PCR — polymerase chain reaction; Ph' chromosome — Philadelphia chromosome.

3'), and consisted from 30 cycles (94 °C – 30 s, 55 °C – 30 s, 72 °C – 1 min). The final product was consistently treated with Klenow fragment (MBI Fermentas) in the presence of 0,2 mM dNTP 15 min at 37 °C and polynucleotide kinase of T4 phage (MBI Fermentas) in the presence of 1mM of ATP 30 min at 37 °C). Then the phenol–chlorophorm purification of the samples was performed according to [7]. Aliquotes of the samples were analyzed in 2% agarose gels (Gibco BRL, USA).

Using T4 DNA–ligase (MBI Fermentas) the purified PCR products were cloned by blunt ends in pUC19 vector (previously restricted with HincII and purified by phenol–chlorophorm method [7]). For that purpose 200–500 ng of PCR products, 20 ng of the vector, ATP–containing buffer and 1 U of T4 DNA–ligase (MBI Fermentas) were added to the mixture with total volume 10 µl. The reaction was carried out for 2 h at 22 °C. Transformation of the competent cells XL1–Blue MRF' Kan (Stratagene) was performed according to [8]. Plasmids from recombinant clones were purified as described in [9]. The initial sequencing of the clones was performed with the use of Blast programme.

Firstly, using reaction of reverse transcription and two-stage PCR the group of patients with CML and ALL with the break in M–bcr domain of *bcr/abl* gene was selected. In particular, the break in this domain lead to the formation of the hybrid p210 BCR/ABL protein. Secondly, the analysis of dbl domain of those patients was carried out by two–stage PCR on cDNA samples using ext1dbl/extr1dbl and ext dbl/extr dbl primers. The reaction products were separated in 2% agarose gels. During electrophoretic analysis in some samples together with full–length amplification products shorter fragments were detected. The alteration of PCR conditions (i.e. increase of tem-

perature of primer's association, decrease of the term of fragment elongation) didn't significantly affect the occurrence of these bars. For further analysis PCR products were cloned in pUC19 vector and the sequencing of obtained recombinant clones was carried out. The analysis of those clones revealed that the changes in the fragments length are caused by deletions in that domain. Such deletions don't alter the reading frame. The localization of deletions in comparison with the structure of full–length dbl domain of *bcr/abl* gene is presented on the Figure. The respective deletion–dependent alterations in p210 BCR/ABL protein are shown in the Table.

It is well known that in 50–60% of CML patients simultaneous expression of p210 *bcr/abl* and p190 *bcr/abl* genes is being revealed [10,11]. One may suppose that the alterations in the spectrum of functional proteins may cause the alterations in cell composition and influence the course of the disease. Such alterations should be caused by mutations in dbl domain of p210 *bcr/abl* gene (and respectively, in p210 BCR/ABL protein), which finally lead to functional similarity with p190 BCR/ABL protein.

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Table. Analysis of dbl domain of BCR/ABL protein from clones obtained from blood samples of patients with CML and ALL

The form of disease	Clone number	The deleted domain, amino acid residues
Case 1 (ALL)	C12	514–733 (220)
	C16	585–620 (36)
	115	556–683 (129)
Case 2 (CML)	2K10	558–712 (155)
	2K19-1	517–573 (57) + 581–734 (154)
	2K19-2	493–660 (168)
	2K23	493–660 (168)
Case 3 (CML)	2L1	582–729 (148)

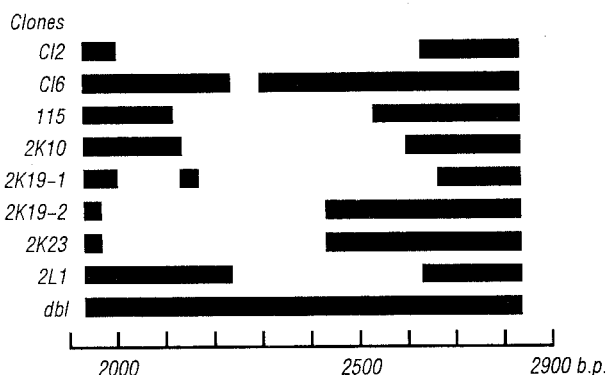


Figure. Localization of the deletions in dbl domain of *bcr/abl* gene (the numbers of clones are on the left). The numeration of nucleotides is presented according to Human *bcr* protein mRNA, 5' end (Genbank, HUMBCRD, Accession: M24603)

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