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Diagnostics of leukemia by biological microchip for analysis of mll translocations

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Abstract. Translocations involving the MLL gene are common in leukemia [1]. MLL plays an important role in the processes of leukemia through the formation of chimeric genes [2, 3], the most frequent of which are MLL-AF4, MLL-ENL, MLL-AF9, MLL-AF10, MLL-ELL, MLL-AF6. Biological microchip research allows for the rapid, accurate and comprehensive detection of MLL gene rearrangements, as well as the precise determination of the fusion point. In this article, we report that biological microarray studies can be used to provide important molecular information that can improve the diagnosis of patients with deukemia. Objective**.** The role of a biological microchip in the analysis of MLL translocations in leukemia.

Keywords: biochip, leukemia, MLL gene, MLL-AF4, MLL-ENL, MLL-AF9, MLL-AF10, MLL-ELL, MLL-AF6.

Introduction. Chromosomal translocations in leukemia, according to recent studies, show that specific chromosome changes can influence the diagnosis and treatment choice for patients [18]. MLL gene rearrangements are among the most common chromosomal abnormalities in acute leukemia. The MLL gene is located at 11q23 and is involved in more than 40 recurrent translocations [19]. MLL rearrangements include unconstitutional deletions, duplications, and mutual translocations [20]. These aberrations usually occur in tumors of certain hematological lines and suggest a decisive role for MLL in determining the disease phenotype or tumor tropism [4]. Up to 80% of acute leukemias in neonates and infants, as well as most secondary leukemias, involve reciprocal 11q23 clonal rearrangement [5,6,7]. The biological microchip allows in one analysis to quickly and accurately identify MLL gene rearrangements among 13 translocations in leukemia. Analysis of leukemia karyotypes in several groups revealed a relative relationship between individual translocations and leukemia subtypes [8,9]. Translocation of the MLL-AF9 gene is detected in AML, and translocation including the AF4 gene occurs almost exclusively in B cell line tumors [4]. The most common partners are AF4 in pro-B ALL and AF9 in AML, mainly M5, accounting for 40 and 27% of 11q23 translocations, respectively. [9]. Rearrangements of MLL genes can occur in AML patients with or without any signs of chromosomal abnormality, resulting in translocation or internal tandem duplication [10,11].

Cytogenetic analysis is a routine part of diagnostic analysis for newly diagnosed and relapsed patients and serves as an independent prognostic factor, providing the basis for stratified approaches to the treatment of these malignancies [12]. However, analysis must be performed on metaphase plates, and a translocation can only be detected and identified if it causes a clear difference in the chromosome binding pattern that renders the analysis unsuitable for routine clinical use. For the rapid detection of MLL rearrangements in acute leukemia, methods of analysis based

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on fluorescence in situ hybridization (FISH) have been described [12,13]. However, these methods cannot be used to accurately identify the partner gene involved. PCR-based methods can be used to detect chimeric RNAs with comparable reliability to FISH, [15] but can only detect MLL gene translocations with a well-defined partner gene and are impractical for identifying all translocations. Fish is usually used to detect chromosomal rearrangements, but requires prior identification of the translocation in order to select the appropriate DNA probe for detection [16]. In addition, PCR assays are sensitive but do not have the ability to multiplex beyond two to four samples due to the complexity of using multiple fluorescent signals [17]. To overcome the internal difficulties in the analysis of complex molecular rearrangements of the MLL gene, an analysis was developed that combines specific amplification of MLL fusion transcripts and identification of the amplified product by hybridization on a biochip.

Materials and research methods. For the study, 25 samples were obtained from peripheral blood cells of patients observed in the Republican Specialized Scientific and Practical Medical Center of Hematology of the Ministry of Health of the Republic of Uzbekistan. The preliminary diagnosis was established on the basis of clinical and laboratory data (morphological and cytochemical). The LK-Biochip set was chosen for the study. Analysis of chromosomal translocations and gene mutations of tumor cells on a biochip included the following steps:

1. Isolation of RNA from peripheral blood leukocytes using the Qiagen RNeasy Mini Kit (USA).
2. Setting up reverse transcription using total RNA isolated from clinical material.
3. Conducting a two-stage "nested" PCR, introduction of a fluorescent label into the PCR product of the second stage using fluorescently labeled primers
4. Hybridization of the labeled PCR product on a biochip.
5. Accounting for hybridization results, image analysis was performed on a device with Imageware software.

The LC biochip contains the following translocation options:

1. (4;11) - fusion of fragments of two genes - MLL (11q23) and AF4 (4q21), occurs in ALL and AML
2. (9;22) p210 - specific marker of chronic myeloid leukemia (CML)
3. (10;11) 3rd place of merger - occurs mainly with M4 and M5
4. (11;19) ENL - initiates monocytic leukemia-like disease
5. (9;11) 1st place of merger - typical for AML, more often M0 or M2 variants
6. (15;17) the place of fusion bcr3 - typical for PLL, AML-MZ, both for the hypergranular form and for the microgranular variant - M3var.
7. (1;19) - found in 1-3% of adult patients with ALL and in 1-6% of children
8. (12;21) j1 fusion site - is the result of the formation of the chimeric oncogene ETV6-RUNX1 (TEL-AML1), found in 25% of children with pre-B-ALL
9. (11;19) ELL - occurs in ALL patients with co-expression of myeloid antigens, observed mainly in AML M4 and M5
10. (inv) inversion 16- fusion site j1 - found in all patients (100%) with a diagnosis of M4 AML

11. (8;21) - typical for AML M4
12. (6;11) - arises from the fusion of the AF6 gene, normally located at 6q27, and the MLL gene, located at 11q23. This chimeric gene is designated MLL-AF6.
13. (9;22) p190 - is an extremely unfavorable factor in the prognosis of ALL

Results and discussion. The studies were carried out on leukemic cells of 25 patients with leukemia, 18 of whom were previously diagnosed with translocations by karyotype. The results obtained with the "LK-biochip" were completely consistent with the results of cytogenetic and PCR analysis. The study revealed 3 clinical samples positive for MLL-AF9 (9;11) (p22;q23), 2 clinical samples were positive for MLL-AF4 t (4;11) (q21;q23), 3 clinical samples were positive for MLL-ENL (11;19), 2 clinical samples were positive for MLL-AF6 (6;11). The efficacy of microarrays in accurately detecting MLL translocations and their fusion partner genes has previously been demonstrated in a blinded study of five leukemic cell lines, 31 MLL-positive and negative patient samples. The result of the study showed a highly specific and highly sensitive methodology. In all cases, the results of the MLL FusionChip were fully consistent with the results of existing methods [21]. The results of this study showed that the analysis can detect the MLL gene regardless of the location of the breakpoint. It is very important to assess the quality of RNA before hybridization, since low quality RNA does not give reproducible results. With the correct selection of the panel of genes responsible for a specific disease, this technique is superior to FISH and Southern blotting, which require several steps to identify the partner gene, and is more reliable than RT-PCR in detecting cases with rare variant transcripts. At the same time, this type of molecular information indicates suitable primers for development for monitoring the disease of patients after treatment [21]. According to some reports, a positive result for MLL FusionChip, indicating MLL-ITD, did not always correlate with internal genome duplication detected by RT-PCR [22]. Several studies have shown that MLL splicing is extremely complex, and several abnormally spliced MLL transcripts have been described in both malignant and normal tissue [23]. In these spliced mismatched products, MLL exons are linked in aberrant genomic orientation (exon scrambling) and are not the result of exon duplication. The ability of MLL FusionChip to detect background events of MLL-ITD erroneous repetition in the transcriptome that are absent in the genome demonstrates the relatively high sensitivity of biochips. Fusion proteins and downstream gene products are potential points of intervention in the treatment of patients with leukemia, and some new molecular technologies have been proposed to target chromosomal translocation products [24]. With the right selection of the genes required to detect leukemia by biological microchipping, it has proven its value and confirmed that molecular strategies such as this can contribute to a new understanding of the diagnosis and follow-up of patients with MLL rearrangements.

Conclusion. These results collectively demonstrate that, with the correct selection of the mutation spectrum, required translocations, the performance and clinical utility of the biological microarray kit is at least equal to or superior to Fish and PCR. The studies carried out on the biochip make it possible to more accurately identify patients with molecular genetic lesions affecting the MLL gene and can be

easily used for the diagnosis and treatment of leukemia. In addition, the introduction of a biochip-based methodology for the diagnosis of patients with primary leukemia has led to increased efficiency in both financial and practical terms [25].

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