APPLICATION OF FISH TECHNIQUE FOR CYTOGENETIC ANALYSIS IN CHRONIC MYELOID LEUKEMIA PATIENTS

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ОПЫТ ПРИМЕНЕНИЯ МЕТОДА FISH ДЛЯ ЦИТОГЕНЕТИЧЕСКОГО АНАЛИЗА У БОЛЬНЫХ ХРОНИЧЕСКИМ МИЕЛОЛЕЙКОЗОМ

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The detection of cytogenetic anomalies is important for the diagnosis and prognosis of hematological malignant diseases. Karyotypes of 32 Armenian patients with chronic myeloid leukemia have been studied for the presence of Philadelphia (Ph) chromosome resulting from translocation t(9;22)(q34;q11) by means of conventional karyotype analysis (CK) and fluorescence in situ hybridization (FISH) in the bone marrow and/or peripheral blood specimens. In our study application of metaphase or interphase FISH in chronic myeloid leukemia patients permitted to confirm and improve the results of conventional cytogenetic analysis.

***Key Words*: cytogenetics, chronic myeloid leukemia, Philadelphia chromosome, FISH.**

Изучение цитогенетических аномалий является необходимым условием для установления диагноза и прогноза гематологических злокачественных заболеваний. Кариотипы 32 больных с хроническим миелолейкозом были исследованы на наличие филадельфийской транслокации t(9;22)(q34;q11) с применением соответствующих методов цитогенетического анализа – стандартной дифференциальной G-окраски (G-бэндинга) и метода FISH (fluorescence *in situ* hybridization) в клетках костного мозга и (или) периферической крови человека. Применение метафазного или интерфазного FISH-анализа позволило уточнить и дополнить результаты цитогенетического анализа даже при наличии метафаз невысокого качества.

***Ключевые слова:* цитогенетика, хронический миелолейкоз, филадельфийская хромосома, FISH.**

Cytogenetic anomalies play an important role in diagnosis of hematological malignant diseases and are important independent predictors of disease progression and survival. The first described chromosomal marker for malignancy in 1960 was so–called Philadelphia chromosome [1]. By application of chromosome banding [2], Philadelphia (Ph) chromosome was due to a translocation t(9;22)(q34;q11). Usually, this translocation brings to the formation of the BCR–ABL fusion gene. Detection of that fusion gene is very important because of leukemogenic role of the BCR–ABL–encoded protein in CML [3]. Thus, Ph translocation serves as a prognostic cytogenetic marker during treatment of CML.

Fluorescence *in situ* hybridization (FISH) technique permits to realise the precise analysis of chromosomal abnormalities in both metaphase and interphase cells. FISH, a technique of molecular cytogenetics, allows the detection of chromosomal loci, which contain genes involved in leukemogenesis [4]. By using FISH–technique BCR–ABL rearrangement can be detected in metaphase spreads of insufficient quality or in interphase nuclei in the case of terminally differentiated cells or of cells which do not divide *in vitro* [5]. Especially important is the ability of interphase–FISH to permit the analysis of interphase nuclei containing BCR–ABL fusion [6].

The goal of the present work was to analyse the presence of Ph chromosome in cells of CML patients in Armenia using FISH technique, and to compare the results with those of conventional karyotyping.

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Abbreviations used: BM — bone marrow; CK — conventional karyotyping; CML — chronic myeloid leukaemia; FISH — fluorescence *in situ* hybridisation; PB — peripheral blood; Ph — philadelphia chromosome.

MATERIALS AND METHODS

Samples. We studied cultured peripheral blood (PB) and/or bone marrow (BM) cells of 32 patients with CML from Hematological Center of Armenia; 28 patients were in accelerated phase, and 4 patients in blast crisis phase. There were 13 men (average age 48.7 ± 5.4 years) and 19 women (average age 44.8 ± 3.9 years).

Conventional cytogenetic study. For cultivation of cells, 10 ml of RPMI 1640 (Sigma, USA) cultural medium with L–glutamine was prepared with the addition of 15% fetal calf bovine serum (BioMedia, Malay–

sia) in 50 ml plastic culture flasks from Cellstar® (USA). Penicilline–streptomycine mix was added to each flask at 100 µg/ml and 100 µl/ml final concentrations (Eurobio, France). 1 ml of PB or BM sample was added to 10 ml of cultural medium and cultivated for 24 and 48 h at 37 °C. Dividing cells were arrested in mitosis with the use of 100 µl or 150 µl of colcemid for PB or BM samples, respectively, for the last 2 h of cultivation. Then hypotonic solutions of KCl were used (0.075 M for PB and 0.15 M for BM samples). The cell fixing, slide preparation and G–banding procedures were performed using standard protocols. Standard cytogenetic analysis was carried out by means of GTG–banding (G–banding by trypsin and Giemsa) on 25 metaphases of each patient, where 5 metaphases were karyotyped.

FISH. For performing the FISH technique, slides were pre–treated for 15 min in 0.01 N HCl/10% pepsin solution (Sigma, USA) at 37 °C. Then chromosomes were fixed in PBS/37% formaldehyde solution at room temperature for 5 min. The slides were dehydrated by washing in 70%, 85%, 100% ethanol solution series, 2 min each. The LSI BCR–ABL extra signal (ES) dual colour translocation DNA probe kit (Vysis, Downers Grove, IL, USA) was used to detect the BCR–ABL gene fusion in both metaphase spreads and interphase nuclei. The spanning ABL probe labelled in Spectrum–Orange is approximately 650 kb extending from an area centromeric of the arginosuccinate synthetase gene to telomeric of the last ABL exon. Application of probe solution and hybridization procedures was performed as described by the manufacturer. Post hybridization washing were performed for 2 min in 0.4 X SSC/0.3% IGEPAL, pH 7.0 at 73 °C and 20 s in 2 X SSC/0.1% IGEPAL, pH 7.0 at room temperature. FISH analysis was performed on cells from 32 patients.

RESULTS

The results are presented in Table. Our data have shown 100.0% coincidence in specimens from 31 patients where both FISH and CK were applied.

In the analysis of PB it composed 58.08 ± 10.5%, and of BM 51.2 ± 21.0%. Six patients (18.75%) were Ph–negative in both CK and FISH analysis.

DISCUSSION

For the first time in Armenia our research group studied cytogenetic changes in BM and PB cells of 32 patients with suspected chronic myeloid leukemia CML by both mentioned techniques.

CML is a clonal bone marrow disease characterised by neoplastic overproduction of granulocytes. In Armenia as in the Western world, CML accounts for approximately one–fourth of all leukemia cases, i.e., an incidence of 1 case per 100,000 persons per year. CML occurs in all age groups, but is more common in people between 40 and 50 years of age. There is no marked sex preponderance.

Our data showed a concordance between FISH results and G–banding analysis. These results demonstrate that FISH analysis can be used when the quality of the metaphases do not allow G–banding analysis or when the number of metaphases is insufficient. In general, application of FISH permitted to increase the capability of cytogenetic diagnosis [7]. FISH is really a sensitive and quantitative method to detect the BCR–ABL fusion gene in both metaphase and interphase cells [8]. It is especially important for cells of patients with leukemia, where the quality of metaphases is often not so good. Thus, interphase FISH is frequently used to monitor the response to therapy in various hematological malignancies [6]. Our data on comparison of results of application of FISH and CK and percentage of cells with Ph chromosomes in BM versus PB specimens confirms the data of the literature on strong correlation between these two specimen sources [9].

There was no correlation between obtained results on Ph+ with the age and sex in the investigated group [10, 11]. We did not reveal extensive deletions of chromosomes 9 and 22 that can arise during disease progression or at the time of the Ph translocation in 10–30% of patients at diagnosis and may confer a worse prognosis [12].

Application of BCR–ABL gene–specific probes made this analysis fast and reliable. Molecular techniques must be used for the analysis of metaphase or interphase nuclei of leukemic patients for precise cytogenetic study.

In conclusion, our data support the clinical diagnosis by revealing the Ph–chromosome in CML patients and improves the prognosis of patients' health. Application of interphase FISH using BCR–ABL probe allows a rapid detection of the Ph translocation in patients with CML.

Table. Results of analysis for the presence of Philadelphia translocation (Ph) by G–banding and FISH in the bone marrow and/or peripheral blood specimens from CML patients

Case	Gender/Age	Culture	Karyotype	FISH	Cells with Ph chr. (%)
1	F/50	PB	Ph+	Ph+	11
2	F/ 54	PB	Ph+	Ph+	94
3	F/34	PB	Ph+	Ph+	10
4	F/43	PB	Ph+	Ph+	23
5	M/47	PB	Ph+	Ph+	41
6	F/19	PB	Ph+	Ph+	46
7	F/73	PB	Ph+	Ph+	40
8	F/65	PB	Ph+	Ph+	45
9*	M/47	PB	Ph+	Ph+	38
10	M/17	PB	–	Ph–	0
11	F/39	PB	Ph+	Ph+	100
12	M/70	PB	Ph+	Ph+	41
13	M/39	PB	Ph+	Ph+	54
14	F/17	PB	Ph–	Ph–	0
15	M/41	BM	Ph+	Ph+	50
16	M/41	PB	Ph+	Ph+	33
17*	M/61	PB	Ph–	Ph–	0
18	M/9	BM	Ph+	Ph+	5
19	F/34	PB	Ph–	Ph–	0
20	F/50	PB	Ph+	Ph+	40
21	F/48	PB	Ph+	Ph+	100
22	M/25	PB	Ph+	Ph+	100
23*	F/46	PB	Ph+	Ph+	75
24	M/61	BM	Ph–	Ph–	0
25	F/54	BM	Ph+	Ph+	86
26	F/34	BM	Ph+	Ph+	15
27	M/48	PB	Ph–	Ph–	0
28	F/19	BM	Ph+	Ph+	100
29	F/61	PB	Ph+	Ph+	75
30	F/43	PB	Ph+	Ph+	4
31	F/43	PB	Ph+	Ph+	68
32*	M/18	BM	Ph+	Ph+	25

* blast crisis; F — female; M — male.

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