ТІРШІЛІКТАНУ
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THE PHILADELPHIA CHROMOSOME AS A PART OF CHRONIC MYELOID LEUKEMIA

Созылмалы миелоидты лейкоз ауыруына шалдыққан қан тапсырушылардың қанының 55 үлгісі зерттелді. Транслокацияның екі нұсқасы қарастырылды t (9; 22). Шынайы уақыт режимінде әдіс ретінде полимераздық тізбектік реакциясы пайдаланылды. 52,73 % зерттеу объектісінен филадельфиялық хромосома анықталды. BCR-ABL транскрипт гені тексерілді.

Изучено 55 образцов крови пациентов с хроническим миелоидным лейкозом. Исследованы два варианта транслокации t (9; 22). В качестве метода использована полимеразная цепная реакция в режиме реального времени. В 52,73 % объектов исследования обнаружена филадельфийская хромосома. Изучен транскрипт гена BCR-ABL.

Chronic myeloid leukemia (CML) is the most frequently diagnosed case of the all chronic myeloproliferative diseases, which are affecting pluripotent stem cells of precursor myeloid, erythroid, B-lymphoid cells and megakaryocytes, also this is one of the common types of leukemia, occurring in 20 % of all cases of leukemia. Nearly a half of all patients usually discover the disease in the period of their higher professional and social activity, when they are between 30 to 50 years old [1, 2].

The characteristic feature of the disease is the fact of the detection of Philadelphia chromosome (Ph-chromosome), or a specific reciprocal translocation, which is occurring between the chromosomes 9 and 22: t (9; 22) (q34; q11). It is well-known that those translocation represents by itself simply the result of the merger of the two genes, BCR-ABL in Ph-chromosome and ABL-BCR 9q. BCR-ABL encodes the formation process of protein with a higher level of tyrosine kinase activity, which is considered as the central mechanisms in the chronic phase of CML. This kind of karyotype change was found in 95 % of CML cases, so, that's why this (9; 22) may be considered as especial sign of CML. Furthermore, Ph-chromosome was found in 25 % of patient cases as the acute lymphoblastic leukemia (ALL) and in 2 % of patient cases as the acute myeloid leukemia (AML) [3, 4]. On this moment the most effective Ph-detection method is the polymerase chain reaction (PCR) [5, 6].

The main purpose of this work is the detecting of the translocation t (9; 22) by the method of real-time PCR («Real-time PCR») in all patient cases, which have chronic myeloid leukemia [7].

Materials and methods

As a material for the research we had take the samples of peripheral blood of these patients, who were on a survey in the hematological department of the regional clinical hospital and also in the children's cancer and blood diseases center of Karaganda. Patients' age ranged from 1 to 60 years old. During the all molecular genetic manipulations we used the reagents of firm «Amplisens».

After the taking RNA from the blood, for the obtaining of cDNA, we were gaining the reverse transcription reaction by the «Tertsik» device. The amplification and the detection of PCR products for a long time were holded in our device «DT-322» (manufactured by «DNA-technology»).

All manipulations with all clinical specimens (RNA picking out, holding the reaction of reverse transcription for the obtaining of cDNA, PCR and the detection of all amplification products) were carried out in the strict accordence with all instructions, which had been attached to all reagents complex and the requirements for PCR laboratories [8].

Results and discussion

For obtaining of the desired fragment we isolated RNA from the peripheral blood cells. The method of allocation of mRNA BCR-ABL from single clinical material is based on the extraction of total RNA. Then the samples were subjected to the reaction of reverse transcription with the using of the enzyme revertazy. Polymerase chain reaction and the detection of the amplification products were carried out by us in real time regime with the using of two mixtures of oligonucleotides: the amplification plot of mRNA chimeric gene M-bcr-abl (p-210), which corresponds to the relevant section of cross-linking genes bcr and abl (b2a2 and b3a2) and a fragment of mRNA splicing gene N-abl, (which is recommended by the working group of «Europe Against Cancer», EAU) as an endogenous internal control (EIC) and the gene-normalizer.

6 variants of translocation t (9;22) with formation of philadelphia chromosome is described [3]. They are represented on the figure 1. Two from them (b2a2 and b3a2) was studied in this research.

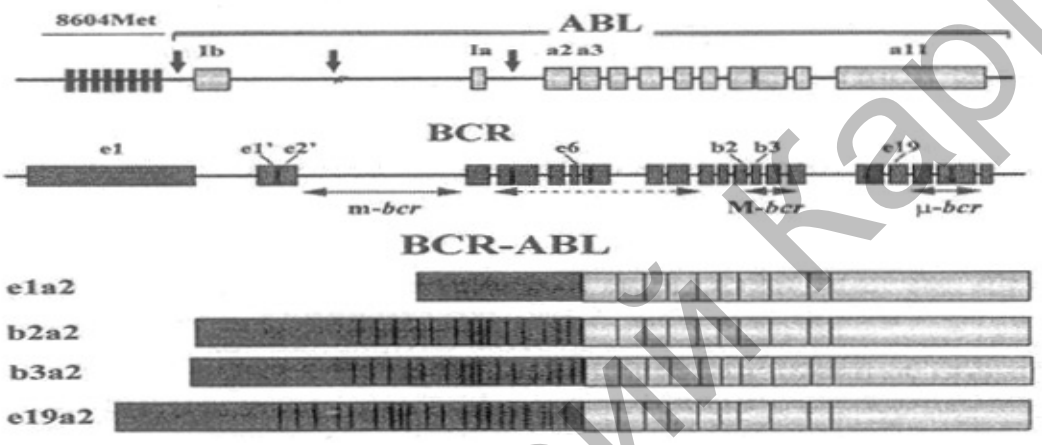

Figure 1. Schematic representation of the ABL and BCR genes disrupted in the t (9;22) (q34; q11)

The presence of the endogenous internal control allows to control the main stages of analysis, including the fence, transporting, storing samples, RNA, carrying out the reaction of reverse transcription of RNA and amplification of cDNA.

During the process of each work stage, we used the positive and the negative control samples (PCS and NCS). For the control in time of the various analysis stages (picking out of RNA and polymerase chain reaction) there were used two types of positive controls: PCS allocation (including the mixture N allocation in the PCR), PCS PCR (including the mixture N allocation in the PCR). The result of amplification of cDNA bcr-abl and abl recordes with the using of HEX fluorescence. The specificity of the reaction proposes the holding of all reactions in two replications. The results of 2 from all 55 tests are showed in Table 1.

Table 1

The results of qualitative analysis of PCR fluorophores HEX

№	Identificator	Ct (Cp)	Concentration
A1	-		-
A2	-	22,0	+
A3	-		-
A4	-	21,8	+
A5	-	25,1	+
A6	-	26,0	+
A7	-	28,6	+
A8	-	38,5	+
B1	-	29,7	+
B2	-	31,9	+
B3	-	25,2	+
B4	-	26,8	+
B5	-		-
B6	-		-

Comment:
A1, A3 — № 1 object's amplification,
A2, A4 — № 1 object's EIC;
A5, A7 — № 2 object's amplification
A6, A8 — № 2 object's EIC.
B, B2 — PCS of isolation (inc. PCR mixture N1),
B3, B4 — PCS of PCR (inc. PCR mixture N2),
B5, B6 — NCS

During the 13 months of 2008 and 2009 we had 55 tests of peripheral blood of patients, who were hospitalized to the hematology department of the regional clinical hospital of Karaganda city and to the Regional Cancer and Blood Diseases Children's Center ambulatory with the possible diagnosis — the chronic myeloid leukemia. During the research of Philadelphia chromosome, we were taking into consideration the different age groups. Due to the work purpose was the determining of the unfavorable environmental factors on the formation of the translocation t (9; 22), we pointed among other factors the place of patient's residence.

All inspection results are presented in Table 2.

Table 2

The patients' data

№	Name, date of birth	Place of residence	Date of giving	Result	Diagnosis
1	2	3	4	5	6
	S. Z., 1966	Karaganda reg., Karkaralynsk dist., Intaly coun.	28.11.08	identified	CML
	M. T., 1936	Karaganda town	03.12.08	identified	CML
	V. V., 1982	Karaganda town	03.12.08	identified	CML
	K. M., 1956	Karaganda reg., Karkaralynsk town	03.12.08	unidentified	CML
	M. S., 1974	Karaganda town	09.01.09	identified	CML, blast crisis
	P. M., 1947	Karaganda town	09.01.09	identified	CML, blast crisis
	A. Z., 1988	Karaganda town	14.01.09	unidentified	CML
	R. S., 1931	Karaganda town	20.01.09	unidentified	CML
	M. I., 1971	Karaganda town	27.01.09	unidentified	CML
	C. T., 1934	Karaganda reg., Temirtau town	27.01.09	identified	CML
	N. V., 1948	Karaganda town	28.01.09	unidentified	CML
	I. I., 1945	Karaganda town	03.02.09	identified	CML
	A. B., 1962	Karaganda town	04.02.09	unidentified	CML
	M. Z., 1937	Karaganda town	24.02.09	identified	CML
	Z. E., 1956	Karaganda reg., Temirtau town	24.02.09	identified	CML
	N. A., 1938	Karaganda reg., Saran town	24.02.09	identified	CML
	K. M., 1951	Karaganda town	03.03.09	identified	CML
	A. K., 1946	Karaganda town	03.03.09	unidentified	CML
	Z. O., 1964	Karaganda town	03.03.09	identified	CML
	T. N., 1932	Karaganda reg., Saran town	03.03.09	unidentified	CML
	P. V., 1936	Karaganda reg., Dolinka vill.	10.03.09	unidentified	CML
	S. A., 1973	Karaganda town	17.03.09	unidentified	CML
	D. T., 1964	Karaganda reg., Balhash town	17.03.09	identified	CML

1	2	3	4	5	6
	M. S., 1977	Karaganda town	19.03.09	unidentified	CML
	S. A., 1952	Karaganda town, Bukhar-Zhyrau dist.	31.03.09	identified	CML
	B. B., 1937	Karaganda reg., Nurinsk dist., Akmechet coun.	07.04.09	identified	CML
	K. V., 1961	Karaganda town	27.04.09	unidentified	CML
	S. U., 1973	Karaganda town	30.06.09	unidentified	CML
	V. M., 1943	Karaganda reg., Bukhar-Zhyrau dist, Petrovka coun.	17.06.09	identified	CML
	K. V., 1937	Karaganda town	17.06.09	unidentified	CML
	O. A.,1957	Karaganda town	29.07.09	unidentified	CML
	C. T., 1934	Karaganda reg., Temirtau town	29.07.09	identified	CML
	H. N., 1981	Karaganda reg., Balhash dist, Konyrat coun.	03.08.09	unidentified	CML
	V. R., 1949	Karaganda town	18.09.09	unidentified	CML
	V. M., 1943	Karaganda reg., Bukhar-Zhyrau dist, Petrovka coun.	21.09.09	identified	CML
	I. K., 1942	Karaganda reg., Karkaralynsk dist., Enbek coun.	07.10.09	unidentified	CML
	Z. B., 1985	Karaganda town	07.10.09	identified	CML
	R. U., 1980	Karaganda town	07.10.09	identified	CML
	A. I., 1936	Karaganda reg., Balhash dist, Konyrat coun.	16.10.09	identified	CML
	S. A., 1924	Karaganda reg., Shahtinsk town	29.10.09	identified	CML
	B. I., 1948	Karaganda town	02.11.09	identified	CML
	U. L., 1949	Karaganda town	05.11.09	unidentified	CML
	D. E., 1938	Karaganda reg., Zhanaarkinski dist., Zhomart coun.	13.11.09	unidentified	CML
	Z. N., 1945	Karaganda reg., Balhash town.	13.11.09	unidentified	CML
	T. S., 1971	Karaganda reg., Bukhar-Zhyrau dist, Gagarin coun.	18.11.09	identified	CML
	E. Z., 1959	Karaganda reg., Karkaralynsky dist.	20.11.09	identified	CML
	Z. A., 1984	Karaganda town, Kazybek-biysky dist.	23.11.09	identified	CML
	Z. T., 1995	Karaganda reg., Oktyabrsky dist., Zhanaarka coun.	25.11.09	identified	Aplastic anaemia
	K. I., 1957	Karaganda reg., Osakarovsk dist., Molodezhny coun.	03.12.09	identified	CML
	Z. A., 1944	Karaganda reg., Temirtau town	03.12.09	unidentified	CML
	C. N., 1977	Karaganda town, Kazybek-biysky dist.	03.12.09	identified	CML
	D. B., 1942	Kagaranda town, Oktyabrsky dist.	07.12.09	unidentified	CML
	A. B., 2008	Karaganda reg.,	08.12.09	unidentified	CML

1	2	3	4	5	6
		Zhezkazgan town			
	S. T., 1937	Karaganda reg., Temirtau town	09.12.09	unidentified	CML
	R. N., 1939	Karaganda reg., Abai dist., Zhartas town	15.12.09	unidentified	CML

As we may see from the table, nearly in 29 of 55 patients' cases, there is the diagnosis of Ph-chromosome. In percentage terms, detected disease takes 52,73 %. At the same time, according to published scientific data, the translocation t (9; 22) usually occurs in 95 % of all cases of CML [3]. It is well-known that the cause of chronic myeloid leukemia may be other variants of this translocation and other chromosomal rearrangements, which are not considered by us in the present work.

Summary results of our studies are represented on the figure 2.

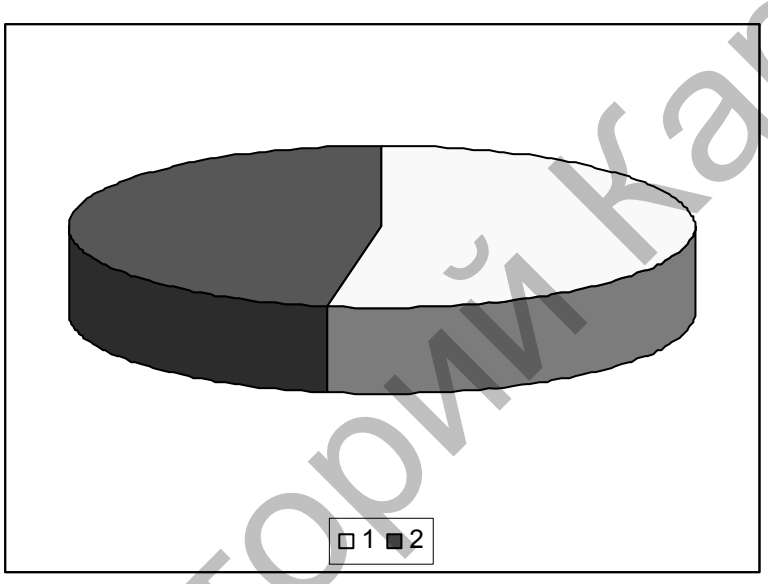

Figure 2. Detection of translocation t (9; 22) *1* — samples without Ph-chromosome; *2* — patients with translocation t (9; 22)

This figure is demonstrated that in 52,73 % of studied samples translocation (9; 22) was detected. Consequently in these cases philadelphia chromosome from the patients with chronic myeloid leukemia was find.

Further researches suggest the scientific review of various forms of leukemia by the other translocation cause malignant tumors method.

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