DETECTION OF *p53* DELETIONS USING FISH VALUES IN THE HL–60, DAUDI, RAJI AND K–562 CELL LINES

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ВЫЯВЛЕНИЕ ДЕЛЕЦИЙ В *p53* МЕТОДОМ FISH В ЛИНИЯХ КЛЕТОК HL–60, DAUDI, RAJI И K–562

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The work is aimed on the analysis of chromosome 17 centromere and *p53* gene-specific signals within interphase and metaphase, determination of *p53* deletion ratios and the detection of the cell-cycle progress. The loss of heterozygosity (LOH) has been investigated using FISH method in 4 cell lines (HL-60, Daudi, Raji and K-562) and peripheral blood samples from 3 Burkitt's lymphoma patients and 2 healthy donors. It is concluded that FISH method may be used in aneuploidy as well as in detection of *p53* deletions and mutations.
***Key Words*: *p53* mutation, cell line, loss of heterozygosity, FISH.**

Проведен анализ центромеры 17-й хромосомы и выявлены делеции в гене *p53* в интерфазе и метафазе. Потеря гетерозиготности была оценена методом FISH в 4-клеточных линиях (HL-60, Daudi, Raji и K-562) и образцах периферической крови 3 пациентов с лимфомой Беркитта и 2 здоровых доноров. Результаты свидетельствуют о том, что метод FISH может быть использован для выявления анеплоидии и делеций/мутаций гена *p53*.
***Ключевые слова*: мутации *p53*, клеточные линии, потеря гетерозиготности, FISH.**

The tumor–suppressor *p53* gene which is known to has been very well preserved throughout the evolution is located on the short arm of chromosome 17 p13. The binding of the p53 to DNA is very important for its tumor–suppressing characteristic [9]. The point mutations of one of *p53* alleles and deletion of the other one result in the loss of tumor–suppressor feature of the gene, and accumulation of various genetic changes that advance the process of carcinogenesis. In fact, more than half of the human cancer cases display mutated *p53* gene. Tumor cells with p53 mutations tend to delete the normal p53 allele and indicate the loss of heterozygosity (LOH) [9].

The *p53* mutations are shown to be frequently observed in Burkitt's lymphoma (BL); moreover, in some BL cell lines, wild type p53 loses its usual function [2]. *p53* mutations have been detected in 33–40% of the sample tissues taken from BL patients and 60–83% of the BL–derived cell lines [7, 8].

The fluorescence *in situ* hybridization (FISH) analysis is a convenient method to analyze huge genetic changes and allows to reveal LOH, aneuploidy, translocation and gene amplification. Furuwatari and al [3] have detected LOH in HL–60 cells using FISH method.

In the present study, FISH method was used to research the presence of LOH in HL–60, Daudi, Raji and K–562 cell lines and BL patients compared to healthy donors.

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Abbreviations used: BL — Burkitt's lymphoma; FISH — fluorescence *in situ* hybridization; LOH — loss of heterozygosity.

MATERIALS AND METHODS

Cell culture and FISH. HL–60, Daudi, Raji and K–562 cell lines were maintained in CO_2 incubator until they reached the log phase and then placed in culture tube. Colchicine was added into each tube (0.5 μg/ml) and the tubes were incubated at 37 °C for 75 min. Hypotonic and fixation procedures were also performed and the cells were then smeared on glass slides.

The peripheral blood samples of 2 healthy persons and 3 BL patients were cultured for 68–72 h in incubator at 37 °C and then harvested with a process that encompassed centrifugation at 1000 rpm for 10 min, addition of a hypotonic solution of 0.075 M KCl and fixation of methanol : glacial acetic acid (3 : 1).

For the FISH analysis, LSI p53, gene–specific DNA probe (spectrum orange) and CEP17 chromosome 17 centromere probe (spectrum green) were used (Vysis, USA).

17p13.1 (p53 locus) and CEP17 hybridized signals were counted in 15 cells from each sample. The LOH was estimated if the FISH value (ratio of the total number of p53 signals to the total number of CEP17 signals) was lower than 0.67 [3].

RESULTS

The FISH method was applied for 9 cell cultures received from 4 cell lines, 3 BL patients and 2 healthy donors used as negative control. In each case, 15 cells per sample were examined. In the control group, the number of CEP17 signals was found to be equal to the number of p53 signals (Fig. 1).

In the HL–60 cell line used as positive control, 2 CEP17 signals and 1 p53 signal were detected in the 13 from 15 cells examined (Fig. 2). The average num–

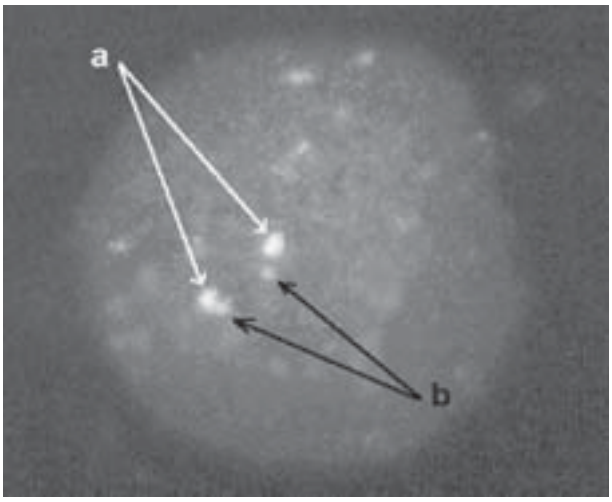


Fig. 1. FISH analysis of the negative control samples: *a)* chromo–some 17 centromere signal (2 signals, white arrows); *b) p53* gene locus (2 signals, black arrows)

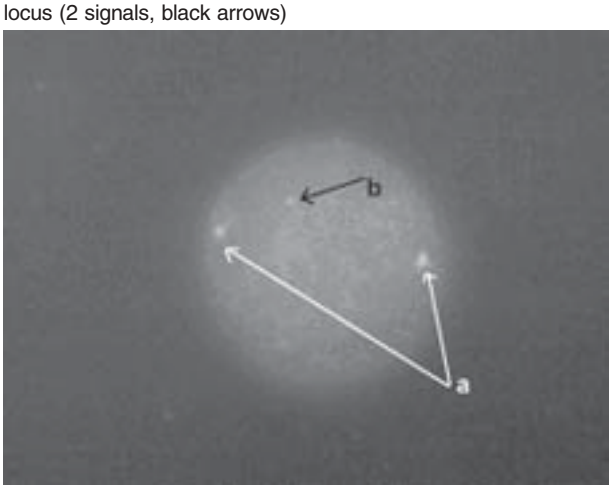


Fig. 2. Loss of heterozygosity of HL–60 cell line (positive cont–rol): *a)* chromosome 17 centromere signal (2 signals, white ar–rows); *b) p53* gene locus (1 signal, black arrow)

ber of chromosome 17 centromere signals in the HL–60 sample was 2.0; however, the average number of p53 signals was 0.86. The HL–60 cells were found to have LOH, since the FISH value was 0.43 (Table).

In the K 562 cells, 4 CEP17 signals were detected in each of the 15 cells examined, and the number of p53 signals remained less than 4 (3 p53 signals were de–tected in 1 cell, 2 — in 12 cells and 1 — in 2 cells). 4 CEP17 signals and 2 p53 signals were shown in Fig. 3. The FISH value for the K–562 cell–line was found to be 0.48, pointing to the existence of LOH (see Table).

On the other hand, the BL–originated Daudi and Raji cell lines did not have LOH, since their FISH va–

Table. FISH values of cell cultures

Cell culture	FISH value*
HL–60	0.43 (0.86/2.00)
Daudi	0.84 (1.8/2.13)
Raji	0.86 (2.00/2.3)
K–562	0.48 (1.9/4.00)
Patient 1**	0.78 (1.66/2.13)
Patient 2**	0.70 (1.6/2.26)
Patient 3**	0.96 (2.13/2.2)
Healthy 1***	1.00 (2.00/2.00)
Healthy 2***	1.00 (2.13/2.13)

* Mean value of the number of *p53* signals / mean value of the number of CEP17 signals.
** Patients treated for BL.
*** Negative control group.

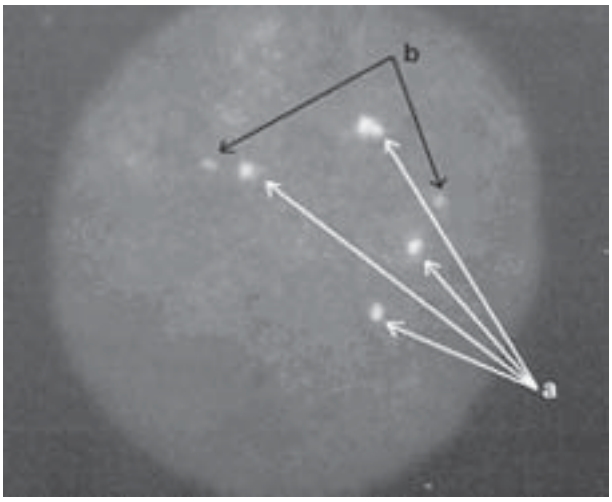


Fig. 3. Loss of heterozygosity of K–562 cell line: *a)* chromo–some 17 centromere signal (4 signals, white arrows); *b) p53* gene locus (2 signals, black arrows)

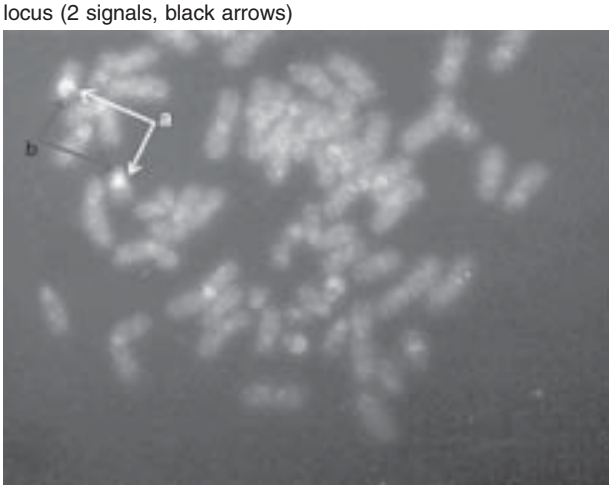


Fig. 4. Daudi cell line during metaphase: *a)* chromosome 17 cen–tromere signal (2 signals, white arrows); *b) p53* gene locus (2 sig–nals, black arrows). Hybridization of *p53* gene probe above chro–mosome 17 centromere signal

lues were found to be 0.84 and 0.86 respectively. Metaphase was observed in both cell–lines (Fig. 4).

Finally, the blood samples of the 3 patients who have received BL treatment indicated no LOH. The FISH value for the first patient, whose treatment continued for 4 months, was found to be 0.78. This value was found to be 0.70 for the second patient who was trea–ted for 3.5 months and 0.96 for the third patient who was treated for 3 months (see Table).

DISCUSSION

It is known that the majority of the somatic cells with p53 mutations have LOH [3]. The great majority of tu–mors with 17p allelic deletions contain mutant p53 genes and similar mutations occur in at least some tumors without 17p allelic deletions [6]. Chromosomal abnor–malities and *p53* gene mutations were detected in the 50% and 10–15% of the lymphocytic leukemia patients respectively [4]. Amiel et al [1] examined 23 patients and 6 healthy donors and by FISH method detected monoallelic p53 deletion in 6 of the 23 patients (26%).

In the present study, the result of the examination of the peripheral blood samples taken from 2 healthy per–sons yielded a FISH value of 1.0 pointing to the absence

of p53 deletion. In the HL–60 cell line, however, the FISH value of 0.43 indicated the presence of LOH that is in accordance with results of other investigators [3]. In 2 cells of the HL–60 line, no signals have been detected for the *p53* gene locus suggesting the existence of homozygosity for the p53. The FISH value was found to be 0.84 for the BL–originated Daudi cell line, which is again close to the value reported by Furuwatari et al [3] as 0.99. Metaphase was observed in this cell line (see Fig. 4). In the human K–562 cell line, p53/CEP17 signals ratio (0.48) points to the existence of p53 deletion (see Fig. 3). In the BL–originated Raji cell line the FISH value was 0.86 indicating no p53 deletion.

The FISH values were found to be 0.78, 0.70 and 0.96 for the patients who received chemotherapy for 4 months, 3.5 months and 3 months respectively (in the last case the desease was characterized by slower rate of progression then in first two cases). One may suppose that those patients have higher chances to have p53 deletions than the individuals in the negative control group for whom the FISH values were 1.0. Therefore, we concluded that BL progression might influence the rate of the p53 deletions. However, the low number of the studied cases didn't allow to made conclusions that are statistically significant.

Since during tumor progression large mutations such as LOH and aneuploidy may arise from the small genetic lesions, FISH analysis is an effective method in their detection [10] and in the monitoring of the BL patients.

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