Allelic loss of selected tumor suppressor genes in acute lymphoblastic leukemia in children

Ewa Studniak[1], Eliza Maloney[2], Tomasz Ociepa[2], Tomasz Urasiński[2], Katarzyna Skonieczka[3], Olga Haus[3], Anna Poluha[4], Jerzy Kowalczyk[4], Stanisław Zajączek[1]

[1]Cytogenetic Unit, Department of Pathology, Pomeranian Medical University, Szczecin, Poland
[2]Department of Paediatrics, Haematology and Oncology, Pomeranian Medical University, Szczecin, Poland
[3]Department of Clinical Genetics, *Collegium Medicum*, Nicolaus Copernicus University, Bydgoszcz, Poland
[4]Department of Paediatrics Haematology, Oncology and Transplantology, Medical University, Lublin, Poland

Defect in function of tumor suppressor genes may lead to initiation/progression of leukemias. *RB1*, *CDKN2A* and *TP53* gene alterations are found in acute lymphoblastic leukemia (ALL) in children. Data showing a contribution of these alterations to the pathomechanism of leukemias are contradictory and their impact on a disease course still remains undefined. The main aim of the study was to identify and the characterize of RB1, CDKN2A and TP53 allele loss in ALL children patients at diagnosis. 46 children with *de novo* ALL were examined. Fluorescent in situ hybridization was performed on bone marrow smear preparations. We demonstrated that at least one of three investigated deletions occurred statistically more frequently in T-lineage leukemia patients ($p = 0.044$); this was the most frequent in respect to *RB1* gene ($p = 0.054$). Additionally, at least one of the examined deletions was observed statistically more frequently in patients with WBC above 20 000/μl ($p = 0.043$), this was the most frequent for *CDKN2A* gene ($p = 0.066$). Presented results seem to give an evidence that deletions of RB1 and CDKN2A genes may contribute to the development of hyperleukocytic type of T-lineage ALL in children, nevertheless this observation needs further investigations.

Key words: RB1, CDKN2A, TP53, ALL, FISH, mono- and bi-allelic loss.

Introduction

RB1, *CDKN2A* and *TP53* gene products play a direct role in cell proliferation control, cell cycle regulation and repair of DNA damage: CDKN2A protein regulates *RB1* and *TP53* pathways, which influence apoptosis directly as well as through cell cycle regulation. Defect in function of suppressor genes deregulates cell growth [1, 2]. *RB1*, *CDKN2A* and *TP53* gene alterations such as translocations, deletions, amplifications or point mutations occur in acute leukemias in children; however, the data that refer to the contribution of these alterations to the pathomechanism of leukemias are repeatedly discrepant and ambiguous, and their impact on the disease course still remains undefined.

RB1 gene deletions as well as their possible effect on the course of a disease have been described in certain leukemias [3-5]. Similarly, coexistence with other high-risk markers was suggested [6, 7]. *TP53* gene alterations have also been observed in a variable percentage of acute lymphoblastic leukemia (ALL) patients. Deletions in the *CDKN2A* gene occur in leukemias in a significant percentage of patients particularly but not only in T-lineage ALL [8-12]. A possible association of these deletions with poor prognosis and minimal residual disease (MRD) was suggested [7, 13, 14]. Detection of minimal residual disease could not be included in this study and requires different investigations.

The data describing alterations in the number of *RB1*, *CDKN2A* and *TP53* alleles in ALL cells are high-

ly inconsistent. We have made an effort to describe the prevalence and the significance of *RB1*, *CDKN2A* and *TP53* allele loss in childhood ALL and its correlation with certain subforms of the disease.

Material and methods

The study group comprised 46 pediatric patients (19 girls and 27 boys aged 8-243 months: mean 98.89 months, median: 90.5 months) with *de novo* ALL diagnosed between 2006 and 2010. Two patients aged 239 and 243 months were included in the study group because of their hospitalization in the Pediatric Department. B-lineage ALL was diagnosed in 35 patients, T-lineage ALL in 11. Bone marrow specimens were obtained in the course of routine diagnostic procedures. We performed fluorescence *in situ* hybridization (FISH) analyses on bone marrow smear preparations obtained from patients prior to the treatment. Bone marrow smears were air dried at room temperature for 1 h, then fixed in freshly prepared acetic acid and methanol (1 : 3) fixative solution for 20 min, and stored at -18æC. All FISH probes were provided by *Vysis*; FISH procedure was performed according to the manufacturer's routine protocol. *LSI 13* (*RB1*) *13q14 Spectrum Orange Probe* was used for the *RB1* gene, *LSI p16 (9p21) Spectrum Orange/CEP 9 Spectrum Green Probe* for the *CDKN2A* gene and *LSI TP53 Spectrum Orange Probe* for the *TP53* gene. Bone marrow smears were analyzed with a fluorescent microscope, concerning each probe, 200 consecutive cells matching the legibility criteria were evaluated. The clinical and laboratory characteristics of patients as well as results of FISH analysis are presented in Table I. The method error for each probe was determined on peripheral blood smears of healthy donors. There were 300 interphase nuclei evaluated per probe. The threshold of error for the cells bearing a deletion was estimated at the level of 0.55% for the *RB1* probe, 0.66% for the *CDKN2A* probe and 0.33% for the *TP53* probe.

In order to test the statistical correlation between discontinuous variables χ^2 Pearson's test was applied. P values < 0.05 were considered statistically significant.

Results

The results of the FISH analysis of *RB1*, *CDKN2A* and *TP53* genes are presented in Fig. 1 and the percentage of cells with deletion is presented in Fig. 2.

RB1 gene

Monoallelic deletions of the *RB1* gene were observed in 11/46 (24%) patients: 6 with B-lineage ALL, 5 with T-lineage ALL. The percentage of cells with the monoallelic deletion was 2%-70% (mean 36%, median 3%). Biallelic deletions of *RB1* were not found. An example of any possible results of the *RB1* gene is presented in Fig. 2.

CDKN2A gene

Deletions of the *CDKN2A* gene were observed in 13/46 (28%) patients: 8 with B-lineage ALL, 5 with T-lineage ALL. The percentage of cells with a deletion was 2%-84% (mean 43%, median 40%). Only in the *CDKN2A* gene, biallelic deletions were observed in 4 patients (1 with B-lineage ALL, 3 with T-lineage ALL). An example of results of the *CDKN2A* gene FISH analysis is presented in Fig. 3.

TP53 gene

Monoallelic deletions of *TP53* were found in 5/46 (11%) patients: 3 with B-lineage ALL, 2 with T-lineage ALL. The percentage of cells with the deletion was 2-20% (mean 11%, median 3%). *TP53* was the only analyzed gene which showed increased (15-70%, mean 42.5%) numbers of copies in 7/46 (15%) patients (6 with B-lineage ALL, 1 with T-lineage ALL). Results of the *TP53* gene FISH analysis are presented in Fig. 4.

Concomitant deletions (presented in Fig. 5) of *RB1* and *CDKN2A* gene copies were seen in 8/46 patients (17%): *CDKN2A* and *TP53* genes in 4/46 patients (9%) and *RB1* and *TP53* deletions in 2/46 patients (4%). Deletions of all three genes, i.e. simultaneous allele loss of *RB1, CDKN2A* and *TP53*, were found in 2/46 patients (4%).

Statistical analysis demonstrated that deletions of the *RB1* gene were more frequent in T-lineage ALL than in B-lineage ALL patients ($p = 0.054$); likewise, coexistence of the *RB1* and *P16* gene deletions occurred significantly more often in patients with T-lineage ALL ($p = 0.044$). *CDKN2A* gene deletion alone ($p = 0.066$), as well as the coexistence of *RB1*, *CDKN2A and TP53* deletions, was more frequently observed in patients with WBC (white blood count) above 20 000/μl ($p = 0.043$).

Discussion

Deletions of the *RB1* gene were observed in 24% of children. According to various authors these alterations are present in 2-38% of patients [15-17]. Discrepancies in findings of different authors may result from various methods employed, distinct leukemia types and differences in ethnicity of studied patients [4, 5, 18, 19]. The percentage of cells showing deletions has not been evaluated in individual ALL patients. The only published study concerned CLL patients, where a high percentage of cells with the deletion correlated with poor prognosis [20].

The phenomenon of allele loss was observed only in ~1/4 of patients, so it may be assumed that it is not involved in the etiology and pathomechanism of the

Table I. Clinical and laboratory characteristics of patients as well as results of FISH analysis

No. of patient	Sex	Age in months	Immuno-pheno-type	Risk group	White blood cell count/μL	Progression-free survival (PFS)	*RB1* gene	*CDKN2A* gene	*TP53* gene
1	F	182	B	IR	1000	57+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
2	M	161	T	IR	113800	57+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
3	F	41	B	IR	47700	7	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A,)×2[200]	nuc ish (TP53×3)[66/200]/ (TP53×4)[100/200]
4	M	48	B	SR	1800	56+	nuc ish (RB1×1)[5/[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
5	M	45	B	IR	29200	38+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
6	M	164	B	IR	2000	36+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
7	M	8	T	HR	9100	48+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
8	F	54	B	SR	9900	46+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
9	M	41	B	HR	15900	47	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
10	M	128	T	IR	57500	46+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
11	M	43	B	SR	7200	43+	nuc ish (RB1×2) [200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
12	M	73	B	IR	11100	42+	nuc ish (RB1 ×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×3)[74/200]
13	F	107	T	IR	457500	37	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×3)[93/200]/ (TP53×4)[30/200]
14	M	16	B	SR	5100	37+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A,)×2[200]	nuc ish (TP53×3)[68/200]
15	M	165	B	HR	11300	36+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
16	M	205	T	IR	55800	NO REMISSION	nuc ish (RB1×1) [10/200]	nuc ish (CEP9×2,CDKN2A×1) [168/200]	nuc ish (TP53×1)[6/200]
17	F	48	B	SR	5200	35+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×3)[120/200]
18	M	56	B	SR	3500	35+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53 ×2)[200]
19	F	96	T	IR	273000	34+	nuc ish (RB1×1)[4/200]	nuc ish(CEP9×2,CDKN2A×1) [106/200]/(CEP9×2,CDKN2A×0) [84/200]	nuc ish (TP53)×2[200]
20	M	101	T	IR	26800	33+	nuc ish (RB1×1)[4/200]	nuc ish (CEP9×2,CDKN2A×1)[4/200]	nuc ish (TP53×2)[200]
21	F	108	T	IR	72900	30+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
22	F	169	B	HR	6800	27+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×3)[140/200]
23	F	57	B	SR	4100	25+	nuc ish (RB1×2)[200]	nuc ish (CEP9×2,CDKN2A×1)[4/200]	nuc ish (TP53×1)[4/200]
24	F	31	B	SR	2700	24+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A×2)[200]	nuc ish (TP53×2)[200]

Table I. Cont.

No. of patient	Sex	Age in months	Immuno-pheno-type	Risk group	White blood cell count/μL	Progression-free survival (PFS)	*RB1* gene	*CDKN2A* gene	*TP53* gene
25	F	165	T	HR	522200	NO REMISSION	nuc ish (RB1×1)[6/200]	nuc ish (CEP9×3,CDKN2A×0)[50/200]/ (CEP9×2,CDKN2A×0)[67/200]/ (CEP9×CDKN2A×0)[65/200]	nuc ish (TP53×2)[200]
26	M	71	T	IR	20100	23+	nuc ish (RB1×2)[200]	nuc ish (CEP9×2,CDKN2A×0)[80/200]	nuc ish(TP53×1)[40/200]
27	M	131	B	IR	3400	22+	nuc ish (RB1×1)[8/200]	nuc ish (CEP9×2,CDKN2A×1)[165/200]	nuc ish (TP53×2)[200]
28	F	139	B	IR	1700	21+	nuc ish (RB1×1)[18/200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
29	F	90	B	IR	12200	21+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
30	F	174	B	IR	21030	44+	nuc ish (RB1×2)[200]	nuc ish (CEP9×2,CDKN2A×1)[140/200]	nuc ish (TP53×2)[200]
31	M	36	B	IR	1350	44+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
32	F	91	B	IR	20140	34+	nuc ish (RB1×1)[140/200]	nuc ish (CEP9×2,CDKN2A×1) [125/200]	nuc ish (TP53×1)[6/200]
33	F	93	B	IR	9570	33+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
34	M	73	B	HR	66930	72+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
35	M	182	B	IR	1760	63+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
36	M	162	B	IR	2250	59+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
37	M	119	B	IR	1410	49+	nuc ish (RB1×1)[4/200]	nuc ish (CEP9×2,CDKN2A×1) [150/200]	nuc ish (TP53×2)[200]
38	F	31	B	SR	14020	48+	nuc ish (RB1×2)[200]	nuc ish (CEP9×2,CDKN2A×1)[12/200]	nuc ish (TP53×2)[200]
39	F	79	B	IR	3510	44+	nuc ish (RB1×2)[200]	nuc ish (CEP9×2,CDKN2A×1)[10/200]	nuc ish (TP53×3)[80/200]
40	M	27	B	SR	4450	60+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
41	F	239	B	IR	3560	66	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
42	M	243	T	HR	62770	40	nuc ish (RB1×1)[120/200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
43	M	129	B	IR	4630	32+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×1)[4/200]
44	M	28	B	IR	32100	35+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
45	M	33	B	IR	40500	39+	nuc ish (RB1×2)[200]	nuc ish (CEP9,CDKN2A)×2[200]	nuc ish (TP53×2)[200]
46	M	67	B	IR	32100	39+	nuc ish (RB1×1)[15/200]	nuc ish (CEP9×2,CDKN2A×1) [91/200]/(CEP9×2,CDKN2A×0) [21/200]	nuc ish (TP53×2)[200]

F – female; M – male; IR – intermediate risk; SR – standard risk; HR – high risk; + – patients remaining alive in remission; nuc ish – nucleus in situ hybridization; CEP9 – centromere of chromosome 9; RB1, CDKN2A, TP53 – tumor suppressor genes; ×0 – biallelic deletion; ×1 – monoallelic deletion; ×2 – two alleles; ×3 – three alleles; ×4 – four alleles; {200} – total number of cells; {40/200} – number of abnormal cells/total number of cells

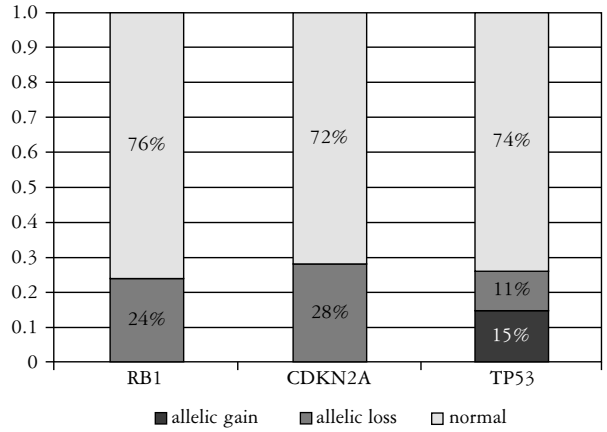

Fig. 1. Results of FISH analysis of RB1, CDKN2A and TP53 genes (number of patients)

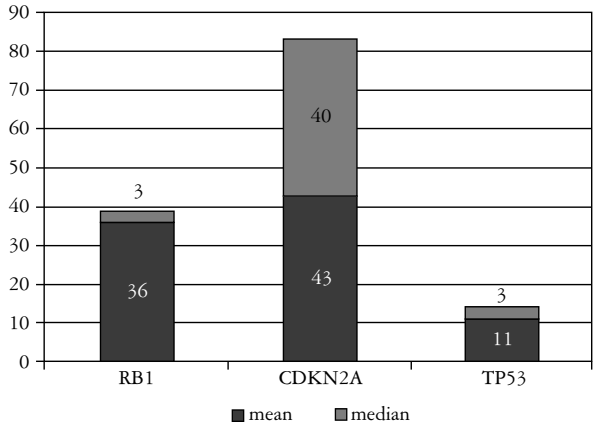

Fig. 2. Percentage (mean and median) of cells with deletion of RB1, CDKN2 and TP53 genes

disease, but it may rather influence the clinical course of leukemia and its prognosis. A possible interpretation of such a mechanism is an impact of *RB1* gene activity on the level of expression of other genes which contribute to regulation of apoptosis and cell proliferation. In the study of Hernandez et al., patients with a high percentage of cells bearing the deletion showed overexpression of genes related to proliferation (*MAPK*, *GRBS*, *RAS*, *SOS1*) and decreased expression of genes which block the cell cycle (*CDKN2C*, *ZAK*, genes of the *GAS* group); low expression of the latter genes led to increased cell proliferation as well. In contrast, in patients with *RB1* deletion, genes which enhance apoptosis (*CASP6, CLU, E2F1*) showed decreased expression, causing a reduction in apoptosis rate and more aggressive clinical course of the disease [20].

Some authors suppose that biallelic deletion of the *RB1* gene is accompanied by loss of protein activity manifested by complete loss of its expression. Thus,

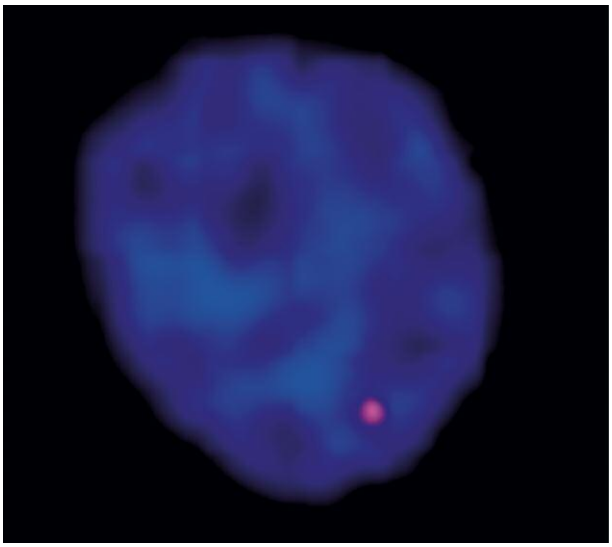

Fig. 3. Results of FISH study using Vysis LSI RB1 probe: one red signal (monoallelic deletion)

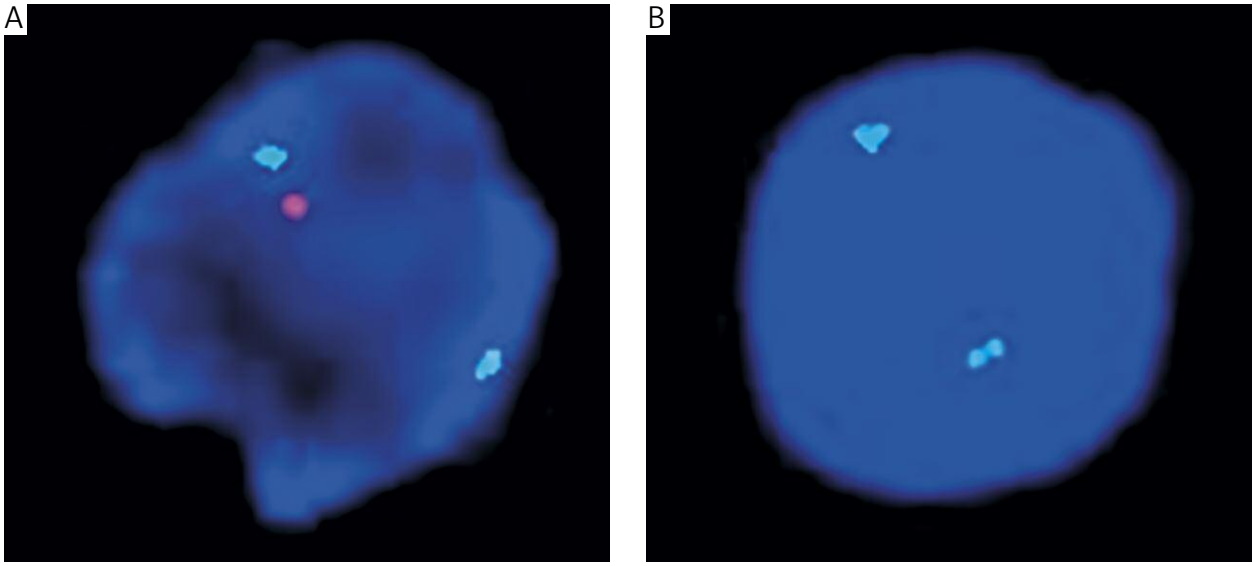

Fig. 4. Results of FISH study using Vysis LSI CDKN2A/P16 probe: **A** – two green control signals and one red signal (monoallelic deletion) **B** – two control green signals and no red signals (biallelic deletion)

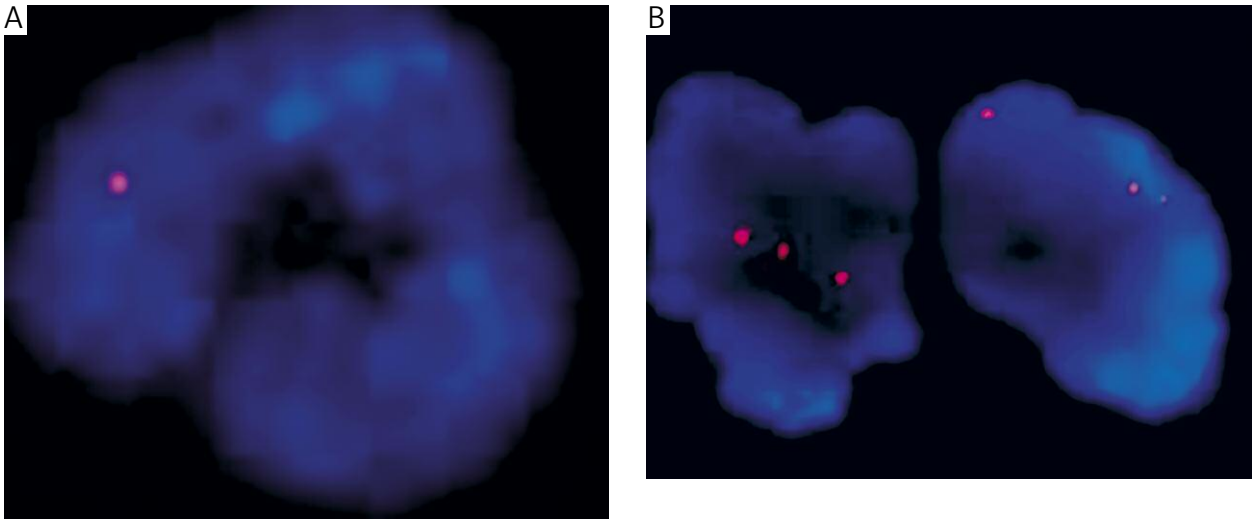

Fig. 5. Results of FISH study using Vysis LSI TP53 probe: **A** – one red signal (monoallelic deletion). **B** – three red signals

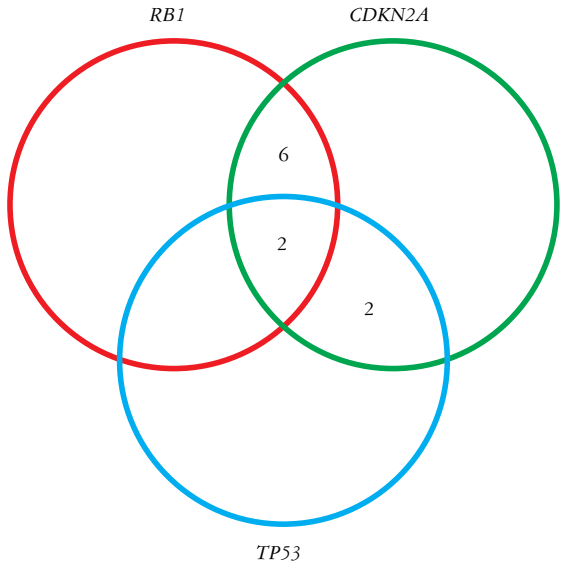

Fig. 6. Number of patients with concomitant deletions of RB1 and CDKN2A genes (8/46), CDKN2A and TP53 genes (4/46 patients), RB1 and TP53 (2/46) and deletions of all three genes (2/46)

a question arises whether decreased gene expression may result from the loss of one allele or, as in retinoblastoma, it has to be a consequence of the loss of both alleles. One of the activity loss mechanisms might be point mutation of the second allele, which is usually undetectable by FISH. In the mechanism of retinoblastoma development, gross deletion, detectable by FISH, usually appears as the second, subsequent somatic mutation, which might be in line with our findings. In the study published by Cheng *et al. RB1* gene expression alterations were found in 18% of patients with different types of ALL [21]. One way to measure the expression of RB1 protein is the level of its phosphorylation. It was shown that the level of RB1 phosphorylation in patients with B-cell ALL is higher prior to the treatment and is declining in response to glucocorticosteroids [22].

Most studies showing loss of *RB1* alleles in leukemic cells have comprised patients with the B-lineage case since it is the predominant form of ALL; however, it was suggested that the alterations in genes which control G1/S phase, such as the *RB1* gene, play an important role only in differentiation of B-lineage but not of T-lineage leukemias. Rearrangements of the *RB1* gene in pre-B ALL were found in 38% of pediatric patients [23]. Other authors have also found *RB1* gene deletion exclusively in B-ALL, in 16% of children and 11% of adults, respectively [17, 18]. Our findings did not support this observation because *RB1* gene deletions were present in both T and B-lineage ALL. This is in line with the study of Paulsson's group, who found *RB1* deletion also in T cell leukemia [24].

CDKN2A allele loss was observed in 28% of patients. In the available literature there is a wide percentage range of children or adult ALL patients bearing these alterations: 8% up to 95% [25, 26]. The various percentages of patients with loss of the *CDKN2A* allele may result from different age of patients. *CDKN2A* deletions were also detected using different technique: ~8-44% of patients (Southern blot), ~17-53% (RT-PCR) and in 31-73% of patients (Q-PCR) [10, 16, 27-29].

In our study monoallelic deletions of the *CDKN2A* gene were observed both in B-lineage and T-lineage ALL patients with the predominance of T-ALL patients, which corresponds to the results of other authors [9, 29]. In the observations of Carter *et al*., proportions of B-lineage and T-lineage ALL patients with *CDKN2A* deletions were equal [30]. One possible reason for such divergence might be the fact that these analyses were done employing different methods and in patients at different age.

CDKN2A is the only one among analyzed genes which also showed biallelic deletions; however, they were found less frequently as compared to monoallel-

ic deletions. Some authors observed both deletion types in an equal percentage of patients [8, 9]. Others found biallelic deletions in a higher number of patients than the monoallelic ones and some authors found biallelic deletions in a lower number of patients [7, 27, 30, 31]. Conversion of monoallelic into biallelic deletion requires additional time; therefore biallelic deletions might be more likely in adults.

In our study biallelic deletions were rather uncommon; however, they were found in 4 out of 11 (27%) patients with T-lineage ALL. It is in line with the findings of Drexler *et al*.: biallelic deletions were observed more often in T-lineage ALL (64%) [32]. It is interesting that, for unknown reasons, in our results, 8/13 patients (61%) with *CDKN2A* allelic loss presented with WBC over 20 0000/μl.

It is supposed by the majority of authors that *CDKN2A* deletions are among possible factors of poor prognosis [11, 28, 32, 33]. However, other reports indicate that this is not a distinct prognostic marker and a poor prognosis results rather from coexistence of other factors [6, 10, 33-35]. Nevertheless, worse prognosis is more often related to the presence of biallelic deletion and a complete lack of CDKN2A protein expression rather than to the presence of monoallelic deletion coexisting independently of mentioned cytogenetic factors. According to the literature, duration of remission was considerably shorter in the group of patients with deletions in comparison to the group of patients without the deletion. Patients with *CDKN2A* deletions also showed notably higher risk of relapse [28, 35]. Kim *et al.* speculate that the presence of biallelic deletion has a negative impact on the overall survival time in adults but not in children [7]. Our observations differ from those of Kim *et al*., since their results were obtained in studies on B-ALL patients, whereas in our study the majority of patients with biallelic deletions had prognostically worse T-lineage ALL. It might be stated that the prognostic relevance of *CDKN2A* gene deletions in children with ALL cannot be defined as yet; however, existing data suggest an impact of *CDKN2A* deletions on prognosis in childhood ALL.

Monoallelic deletions of the *TP53* gene were found in 11% of patients; thus they do not seem to be, like *RB1* and *CDKN2A* deletions, a factor initiating ALL. Using FISH, Aqirre *et al.* detected *TP53* deletions in 7% of patients with ALL [36]. This is in line with our results confirming a much lower incidence of *TP53* deletions as compared with those of *RB1* or *CDKN2A.* The low percentage of cells with *TP53* deletion in ALL patients as well as the resistance of ALL cells to apoptosis reported in the literature suggests that apoptosis in ALL might also be regulated by mechanisms disturbing *TP53* gene function other than these deletions [37].

In our study, *TP53* deletion was found in 3/35 (9%) patients with B-lineage ALL and in 2/11 (18%) patients with T-ALL. To our best knowledge, data on the frequency of *TP53* deletion in children with ALL have not been reported. The only available data on this issue indicate that the frequency of point mutations in children with B- and T lineage ALL varies from 2 to 5% and from 5 to 50% respectively [12].

The mean number of cells with loss of the *TP53* allele was the lowest in comparison to other analyzed genes (11%). According to our best knowledge the rate of cells showing *TP53* deletions in patients with ALL has not been evaluated. Lazaridou *et al*. observed loss of one allele of the *TP53* gene in 29% of CLL patients with 28-98% of cells bearing this deletion. Alterations of *TP53* occurred in the majority of patients at clinical presentation [38]. The deletions of both alleles of the *TP53* gene were not found in our patients, but it does not exclude the possibility of the loss of function of both alleles. It may be analogous to the loss of heterozygosity (LOH), i.e. coexistence of both deletion of one allele and point mutation of the homologous one. This phenomenon was described by Wattel *et al*., who detected 17p deletion coexisting with missense mutation of the second *TP53* copy in half of ALL patients, while the point mutation alone was found in remaining cases [39]. These observations seem to indicate that coexistence of deletion and point mutation of *TP53* may have a different functional impact than the gene deletion alone [40].

Conclusions

1. *RB1*, *CDKN2A* and *TP53* gene deletions are found in substantial proportions of children with ALL, occurring with a various frequency.
2. The results of our study seem to indicate that deletions of *RB1* and *CDKN2A* cancer suppressor genes may contribute to the development of hyperleukocyte types of T-lineage ALL in children; nevertheless, this observation as well as assessment of its prognostic significance needs further investigation.

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Address for correspondence

Ewa Studniak PhD
Cytogenetic Unit, Department of Pathology
Pomeranian Medical University
Polabska 4
70-115 Szczecin, Poland
tel./fax +48 91 466 15 45
e-mail: ewa.studniak@gmail.com