# Abnormalities of the *P53, MDM2, BCL2* and *BAX* genes in acute leukemias

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Abnormalities of the P53 network have been implicated in the pathogenesis of acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML). The purpose of this study was to define *P53* gene mutations, to detect *MDM2* gene amplification and to estimate mRNA expression of *P53*, *MDM2*, *BCL2* and *BAX* genes in patients with ALL and AML. Twenty-five patients with ALL and 65 patients with AML, both recently diagnosed, were included into this study. Exons 5-8 of the *P53* gene with flanking intronic sequence were amplified by the polymerase chain reaction (PCR) method and subjected to mutation screening by single-strand conformation polymorphism analysis (SSCP). Mutation of the *P53* gene was found in one patient of the 25 with ALL and in five patients of the 65 with AML. Sequence analysis was subsequently performed. One mutation in intronic sequence in ALL and four missense mutations and one silent nucleotide substitution in AML were identified. Amplification of *MDM2* gene was detected by multiplex-PCR analysis in only one sample from patient with ALL, but was not observed in any case of AML. To gain further insight into the role of P53 network in the evolution of acute leukemias, the *P53*, *MDM2*, *BCL2* and *BAX* mRNA expressions in a portion of samples from patients with ALL and AML were analyzed using multiplex RT-PCR. Although a low frequency of molecular disturbances of the *P53* and the *MDM2* genes was detected in this study, there was a high percentage of cases with increased mRNA level of *P53* and *MDM2*. A high frequency of *BCL2* mRNA overexpression and a relatively low frequency of *BAX* mRNA overexpression detected in both analyzed leukemias in this study, indicate that altered transcription of these genes may be involved in leukemogenesis.

*Key words: P53, MDM2, BCL2, BAX, ALL, AML*

The *P53* tumor-suppressor gene integrates numerous signals that control normal cell proliferation and apoptosis [33]. The P53 protein functions as a transcription factor by binding to specific DNA sequences and regulating the transcription of target genes involved in cell cycle control, DNA repair and apoptosis [17]. In response to DNA damage and other forms of stress the P53 promotes either cell cycle arrest at the $G_1$/S or $G_2$/M transitions or apoptosis [2]. Under physiologic conditions, the functional activity of P53 is negatively regulated by the MDM2 oncoprotein through direct physical association, while P53 increases MDM2 at the transcriptional level [16]. The activity of P53 and the expression of MDM2 are coregulated in autoregulatory feedback loop [30]. There is evidence that P53 downregulates the expression of the anti-apoptotic gene *BCL2* and upregulates the expression of the pro-apoptotic gene *BAX* [19]. Mutation of the *P53* tumor-suppressor gene is one of the most common molecular alterations in a variety of tumors [12], but it occurs infrequently in ALL (5%) [14] and AML (9%) [31]. Mutations of the *P53* gene often cause changes in the conformation of the P53 protein and lead to its inactivation [10]. In about half of human tumors, P53 is inactivated directly as a result of mutations in the *P53* gene. An additional proposed mechanism for *P53* transcript accumulation and inactivation of P53 protein is the amplification of the *MDM2* and its overexpression [6].

The amplification of the *MDM2* oncogene and resulting from this overexpression of MDM2 protein have been demonstrated in a high percentage of human soft tissue sarcomas [20]. Overexpression of *MDM2* mRNA has been reported in 42% of ALL and 53% of AML, but in the absence of *MDM2* gene amplification [3]. Some studies have shown a strong

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correlation between MDM2 protein overexpression and P53 protein overexpression in some human carcinomas [5, 7]. This supports the idea that one of consequences of overexpression of MDM2 may be the elimination of P53 tumor suppressor function.

*BCL2* and *BAX* are members of the BCL2 family of apoptosis-regulating genes [27]. The anti-apoptotic function of the BCL2 protein is modulated by its ability to heterodimerize predominantly with BAX, a protein favoring induction of apoptosis. Cell survival or death following an apoptotic stimulus may, therefore, be dependent on the ratio of BCL2 to BAX [25]. It has been shown that an abundant expression of BCL2 may block P53-dependent apoptosis [36]. High expression levels of BCL2 have been shown to correlate with poor treatment in some hematological malignancies, including follicular lymphoma, chronic lymphocytic leukemia and also in acute myeloid leukemia [11, 15, 28]. The loss of BAX expression is a frequent event in patients with solid tumors and a negative prognostic factor for therapeutic response [32]. It has been reported that decreased BAX expression is associated with an increased risk of relapse of childhood acute lymphoblastic leukemia [26].

In order to elucidate the above controversial data the investigation of *P53* gene mutations, *MDM2* gene amplification and estimation of mRNA expression of *P53*, *MDM2*, *BCL2* and *BAX* genes in samples of patients at initial diagnosis of acute leukemia were performed in this study.

## Patients and methods

The study included 90 patients with acute leukemias (25 ALL: 7 female, 18 male, median age 24, range 17–57 years; 65 AML: 36 female, 29 male, median age 60, range 18–81). The patients were diagnosed in the Department of Hematology, Medical University of Lodz, between 1998 and 2001. The groups of ALL patients consisted of the following immunological subgroups found: 3 pro-B cell ALL, 18 common ALL, 1 pre-B cell ALL, 1 pre-T cell ALL and 2 T cell ALL. The groups of AML patients consisted of the following FAB subtypes found: 1 M0, 12 M1, 29 M2, 5 M3, 15 M4 and 3 M5. Peripheral blood or bone marrow samples were obtained from the patients prior to the initiation of therapy.

Genomic DNA from the peripheral blood or the bone marrow blast cells was isolated by lysis of the cells with sodium dodecyl sulphate (SDS), digestion with proteinase K at 37 °C overnight followed by phenol/chloroform extraction and ethanol precipitation. Four genomic regions of *P53* gene (exons 5–8) with flanking intronic sequence were amplified by PCR using four sets of 20 bp primers, which were synthesized according to the published sequences of *P53* [24]. The sequences used for the primers are summarized in Table 1. PCR was performed in a final volume of 20 µl containing 50 ng DNA, 0.5 µM of each primer, 50 µM dNTPs, 1.5 mM $MgCl_2$ and 0.5 U of Taq DNA polymerase (Promega). After 5 min at 94 °C, 35 cycles of amplification, consisting of 60 s at 94 °C, 60 s at 58–61 °C and 60 s at 72 °C, were performed, with a subsequent 7 min extension at 72 °C.

For the SSCP analysis, PCR was performed by adding 1 µl of [-$^{32}$P]dCTP (370 MBq/ml) to the PCR mixture. After amplification, labeled PCR products were diluted 1:5 in 95% formamide, heated to 94 °C, chilled on ice, and immediately loaded (3 µl) onto a 6% neutral polyacrylamide/TBE gel containing 10% glycerol. Electrophoresis was performed at 40 W for approximately 4 h at 4 °C. The gel was then dried on filter paper and autoradiography was performed.

PCR products that demonstrated an aberrant migration pattern in the SSCP autoradiograph compared to wild-type gene were analyzed for determination of nucleotide sequence. PCR was performed in a final volume of 40 µl containing 100 ng DNA and 1 U of Taq DNA polymerase (Promega). The PCR products were separated from excessive oligonucleotides and dNTPs by glycogen and isopropanol precipitation. Sequence analysis was performed by the dideoxy termination method using SequiTherm Excel DNA Sequencing Kit (Epicentre Technologies) and fluorescent-labeled primers. The sequences of the labeled primers are shown in Table 2. Products of sequencing reaction were visualized and analyzed using a LiCor automated laser fluorescence sequencer.

A coamplification of *MDM2* and *DR* genes was performed to detect *MDM2* gene amplification in DNA isolated from ninety patients with acute leukemia. PCR was performed in a final volume of 20 µl containing 100 ng DNA, 0.5 µM of each primer for *MDM2* gene, 0.25 µM of each primer for *DR* gene, 100 µM dNTPs, 1.5 mM $MgCl_2$ and 0.5 U of Taq DNA polymerase (Promega). The complementary primer pairs [13] used for multiplex-PCR are listed in Table 3. After 5 min

**Table 1. Sequences of primer pairs for amplification of the exons of *P53* gene**

| |
|---|
| P53 exon 5, 178-bp |
| forward |
| 5'- TTCCACACCCCCGCCCGGCA -3' |
| Reverse |
| 5'- ACCCTGGGCAACCAGCCCTG -3' |
| P53 exon 6, 213-bp |
| forward |
| 5'- ACAGGGCTGGTTGCCCAGGG -3' |
| Reverse |
| 5'- AGTTGCAAACCAGACCTCAG -3' |
| P53 exon 7, 208-bp |
| forward |
| 5'- ACTGGCCTCATCTTGGGCCT -3' |
| reverse |
| 5'- GTCAGAGGCAAGCAGAGGCT -3' |
| P53 exon 8, 227-bp |
| forward |
| 5'- TAAATGGGACAGGTAGGACC -3 |
| reverse |
| 5'- TCCACCGCTTCTTGTCCTGC -3' |

at 94 °C, 30 cycles of amplification, consisting of 60 s at 94 °C, 60 s at 53 °C and 60 s at 72 °C, were performed, with a subsequent 7 min extension at 72 °C. The PCR products were separated by 150 V electrophoresis on 2% agarose gels. The copy number of the *MDM2* gene was compared with reference housekeeping *DR* gene copy number using the system Gel Doc1000 Bio Rad Image with the program Molecular Analyst applied.

Total cellular RNA was isolated from the peripheral blood or the bone marrow samples obtained from fourty recently diagnosed patients (13 with ALL and 27 with AML) using a commercial kit (A&A Biotechnology, Poland) according to the manufacturer's protocol. Total RNA was also isolated from the peripheral blood samples obtained from ten normal donors serving as controls. Contaminating residual genomic DNA removed by digestion with Rnase-free Dnase (Roche). Approximately 1–2 μg of RNA was reverse transcribed into single-strand cDNA in a final volume of 20 μl containing 50 mM DTT, 1.5 μg oligo(dT), 0.5 mM dNTP, 20 units RNase inhibitor and 200 units M-MLV reverse transcriptase (Promega). First-strand cDNA synthesis reaction was performed at 37 °C for 90 min. Estimation of *P53, MDM2, BCL2* and *BAX* mRNA level was examined using the multiplex RT-PCR assay. The following multiplex RT-PCR reactions were performed: 4 μl of the RT-solution, 0.5 μM of each primer for analyzed gene, 0.25 μM of each primer for β-*actin* gene, 100 μM dNTP, 1.5 mM $MgCl_2$ and 0.5 units Taq-DNA polymerase, final volume of 20 μl. The primers were synthesized according to the published sequences [1, 3, 21, 29, 35]. The sequences of primer pairs are shown in Table 4. To estimate the integrity and amount of cDNA, the β-*actin* was amplified with each of the analyzed genes in the same tube as an internal control. After 5 min at 94 °C, 35 cycles of amplification, consisting of 60 s at 94 °C, 60 s at 56–64 °C and 60 s at 72 °C, were performed, with a subsequent 7 min extension at 72 °C. The PCR products were resolved on 2% agarose gels. The *P53*, *MDM2*, *BCL2* and *BAX* mRNA levels were calculated as a relative ratio of particular analyzed genes to β-*actin*.

The correlations between mRNA level of *P53* and mRNA level of *MDM2*, *BCL2* and *BAX* genes were assessed by the Spearman two-sided test. The Kruskal-Wallis test was used to check the heterogeneity of variables among three groups (control group: normal donors, patients with ALL and patients with AML) defined by the level of mRNA of analyzed genes.

## Results

PCR-amplified DNA products of exons 4–8 of the *P53* gene were subjected to the screening of point mutations by SSCP analysis. Mobility shifts were detected in one of the 25 (4%) patients with ALL and in five of the 65 (8%) patients with AML. Figure 1 shows representative results of the SSCP analysis. Sequencing was performed for the six cases with

**Table 2. Sequence of fluorescent-labeled primers for sequencing of the *P53* gene**

| |
|---|
| P53 exon 5 |
| forward |
| 5'- GTGCAGCTGTGGGTTGATTC -3' |
| P53 exon 6 |
| forward |
| 5'- GCCTCTGATTCCTCACTGAT -3' |
| P53 exon 7 |
| forward |
| 5'- ATCTCCTAGGTTGGCTCTGA -3' |
| P53 exon 8 |
| forward |
| 5'- CTCTTTTCCTATCCTGAGTA -3' |

**Table 3. Primer pairs for *MDM2* gene amplification in DNA**

| |
|---|
| MDM2, 143-bp |
| Forward |
| 5'- GAGGGCTTTGATGTTCCTGA -3' |
| Reverse |
| 5'- GCTACTAGAAGTTGATGGC -3' |
| DR, 113-bp |
| Forward |
| 5'- CCACTGAATCTGTCCTGGTATG -3 |
| Reverse |
| 5'- GTGTGGCATAGTAGTTGTAGTGG -3' |

**Table 4. Primer pairs for amplification *P53, MDM2, BCL2, BAX* and β-actin mRNA**

| |
|---|
| P53, 455-bp |
| Forward |
| 5'- CAGCTCCTACACCGGCGGCCCCTGCACCAG-3' |
| reverse |
| 5'- GAGCCAACCTCAGGCGGCTCATAGGGCACC-3' |
| MDM2, 335-bp |
| Forward |
| 5'- TGAAGGTTTCTCTTCCTGAAG -3' |
| reverse |
| 5'- TTATTAAAGTCTGTTGGTGCA -3' |
| BCL2, 389-bp |
| Forward |
| 5'- ACTTGTGGCTCAGATAGGCACCCAG -3' |
| reverse |
| 5'- CGACTTCGCCGAGATGTCCAGCCAG -3' |
| BAX 538-bp |
| forward |
| 5'-CAGCTCTGAGCAGATCATGAAGACA-3' |
| reverse |
| 5'-GCCCATCTTCTTCCAGATGGTGAGC-3' |
| β-actin (1) 548-bp |
| Forward |
| 5'-GTGGGGCGCCCCAGGCACCA-3' |
| Reverse |
| 5'-CTCCTTAATGTCACGCACGATTTC-3' |
| β-actin (2) 838-bp |
| Forward |
| 5'-ATCTGGCACCACACCTTCTACAATGAGCTGCG-3 |
| Reverse |
| 5'-CGTCATACTCCTGCTTGCTGATCCACATCTGC-3' |

mobility shifts. One mutation in ALL was located in intron 7; four missense mutations and one silent nucleotide substitution in AML were located in exons 6–8. No mutations were identified in exon 5. The results of P53 point mutations are summarized in Table 5 and an exemplary result of sequencing analysis is shown in Figure 2.

Four of the six patients with *P53* gene mutations died (patient no. 1, 4, 5 and 6, Tab. 5). Patient no.1 with an intronic mutation of T to A at position 14146 died 11 months after diagnosis. Patients no.4, 5 and 6 died within 1 month after diagnosis without achieving complete remission. Patient no. 2 with a mutation of CGT (Arg) to GGT (Gly) at codon 202 had a relapse after three years, while patient no.3 with a silent mutation had a good response to therapy. These six patients did not have a family history suggesting Li-Fraumeni syndrome. Detection of *MDM2* gene amplification was performed using multiplex-PCR analysis. On the basis of results from normal donors it was established that the analyzed cases show *MDM2* gene amplification when the ratio of intensity of *MDM2* band to the intensity of *DR* band exceeds 0.95 (X+2SD). The criterion of *MDM2* gene amplification was fulfilled in only one sample from patient with ALL (Fig. 3). No gene amplification was detected in any cases of AML.

The relative level of *P53, MDM2*, *BCL2* and *BAX* mRNA in cases of leukemia and in normal leucocytes was established by RT-PCR and coamplification of the analyzed gene and the β-*actin* mRNAs. The *P53*, *MDM2*, *BCL2* and *BAX* amplification products of all normal samples constituted 20% or less of the β-*actin* amplification products. Therefore, the level of mRNA was considered as increased when the ratio of particular analyzed gene to β-*actin* was greater than 20%. Representative results for multiplex RT-PCR analysis are shown in Figure 4. The increased mRNA level of *P53* was detected in 61% cases of ALL and 41% cases of AML, whereas the increased transcript level of *MDM2* was detected in 31% of ALL and in 33% of AML. No statistically significant association between mRNA level of *P53* and mRNA level of *MDM2* was detected (the Spearman two-sided test; p=0.2303 in ALL and p=0.1445 in AML). Overexpression of *BCL2* mRNA was detected in 77% of ALL and in 41% of AML, whereas overexpression of *BAX* mRNA was detected in 46% of ALL and in 22% of AML. No statistically significant correlation between mRNA level of *P53* and *BCL2* (p=0.4225 in ALL and p=0.0534 in AML) and between mRNA level of *P53* and *BAX* (p=0.2711 in ALL and p=0.0868 in AML) was found. Using the Kruskal-Wallis test a significant heterogeneity among three groups (0=control group; 1=patients with ALL; 2=patients with AML) with respect to mRNA level of *P53* (0 vs 1, p=0.0062; 0 vs 2, p=0.0097), *MDM2* (0 vs 1, p=0.0035; 0 vs 2, p=0.0025) and *BCL2* (0 vs 1, p=0.0026; 0 vs 2, p=0.0092) was observed. There was no significant heterogeneity with respect to mRNA level of *BAX* (0 vs 1, p=0.0511; 0 vs 2, p=0.3261).

**Table 5. Types of mutations of *P53* in ALL and AML**

| Patients | Type | Sex/age | Location of mutation | Type of mutation | Amino acid change |
|---|---|---|---|---|---|
| 1 | c-ALL | M/36 | Intron 7, position 14146 | T to A | |
| 2 | AM1L | M/51 | Exon 6, codon 202 | C to G<br>CGT to GGT | Arg to Gly |
| 3 | AM2L | M/45 | Exon 6, codon 213 | A to G<br>CGA to CGG | Arg to Arg |
| 4 | AM2L | F/61 | Exon 7, codon 245 | G to A<br>GGC to AGC | Gly to Ser |
| 5 | AM1L | F/72 | Exon 8, codon 266 | G to A<br>GGA to GAA | Gly to Glu |
| 6 | AM2L | F/65 | Exon 8, codon 284 | A to C<br>ACA to CCA | Thr to Pro |

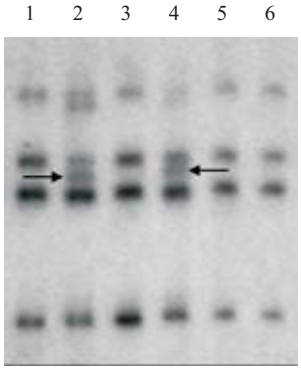


**Figure 1. Examples of PCR-SSCP analysis of the *P53* gene. PCR-SSCP analysis of exon 6. Lane 1, normal leukocytes (negative control), lanes 2–6, samples of AML patients. Mobility shifts were detected in lane 2 and 4.**

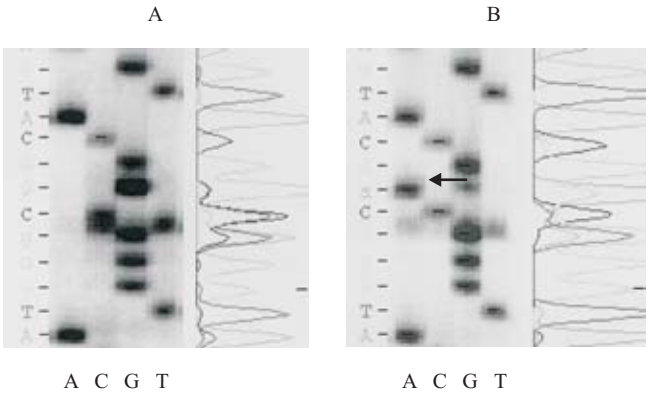


**Figure 2. Sequence analysis of the *P53* mutations. (A) – the wild type of sequence is shown (control); (B) – a fragment of exon 7 sequence with a mutation. A point mutation was detected at codon 245 resulting in a change from GGC (Gly) to AGC (Ser).**

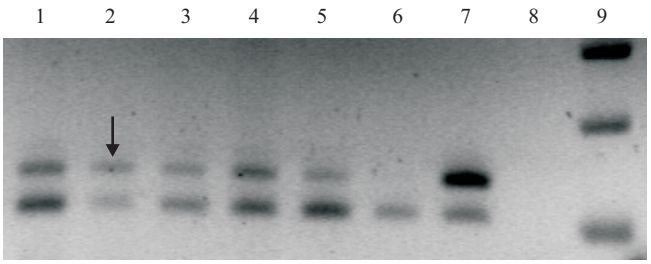


**Figure 3. Multiplex-PCR analysis. Lanes 1–3, samples of ALL patients; lanes 4–5, samples of AML patients; lane 6, negative control (normal leukocytes); lane 7, positive control (sample from glioma tissue); lane 8, negative control (water instead DNA); lane 9, molecular weight marker (100 bp ladder). *MDM2* gene amplification were detected in lane 2.**

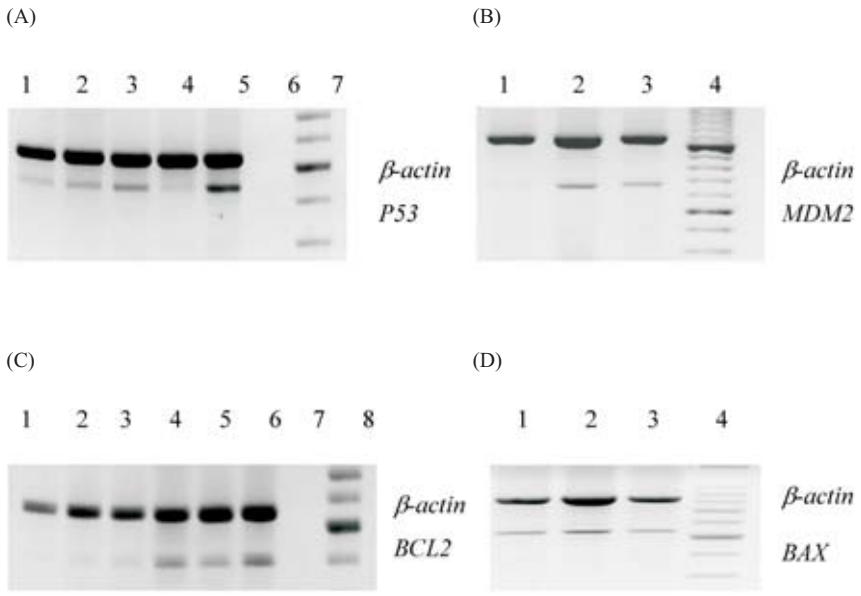


**Figure 4. Multiplex RT-PCR analysis of *P53, MDM2, BCL2* and *BAX* transcripts in representative samples of ALL and AML patients. (A) – *P53* mRNA expression. Lanes 1–5, samples of AML patients; lane 6, negative PCR control (water instead of DNA). In samples 2, 3, 5 the increased mRNA level of *P53* was detected. Ratio of *P53* gene to *β-actin* in these samples is greater than 20%. Sample 5 disclosed also a mutation in *P53* gene. (B) – *MDM2* mRNA expression. Lanes 1–3, samples of ALL patients, 2 and 3 demonstrate increased mRNA level of *MDM2*. Sample 2 shown also amplification of *MDM2* gene. (C) – *BCL2* mRNA expression. Lanes 1–6, samples of ALL patients; lane 7, negative PCR control (water instead of DNA). In samples 4–6 the increased mRNA level of *BCL2* was demonstrated. (D) – *BAX* mRNA expression. Lanes 1–3, samples of AML patients, 1 and 2 demonstrate increased mRNA level of *BAX*.**

## Discussion

The P53 network is potentially disturbed by *P53* gene mutation or *MDM2* amplification in a wide variety of human cancers. A mutation of the *P53* gene is an uncommon event at diagnosis in ALL and AML [14, 22, 31, 34]. Higher incidence of *P53* mutation has been reported in relapsing ALL [38]. These findings suggest that *P53*-mutated minor subclone, which is not detected by PCR-SSCP may mediate relapse by clonal expansion in ALL. A higher incidence of *P53* mutation has been also reported in ALL of FAB L3 subtype and in AML with monosomy of 17p [4, 23]. Most of the P53 mutations in cancer occur in exons 5–8; mutations in other regions are very exceptional, but may play a role in the development of cancer [9]. Intronic mutations leading to defects in activity of P53 protein have been detected in familial breast cancer [18].

In the present study *P53* gene mutations were found in only one of 25 (4%) patients at initial diagnosis of ALL and in 5 of 65 (8%) patients at initial diagnosis of AML. These mutations were located in the coding sequence of exons 6–8 and one in intron 7. Almost all these cases, except one with missense mutation, showed increased level of *P53* mRNA. The case revealing intronic mutation showed also an increased level of *P53* mRNA, suggesting that intronic mutations may influence transcriptional activity of *P53* gene. Several base substitutions at various sites in intron 7 of the *P53* gene have been described previously [8]. In this study the change at nucleotide 14146 in intron 7 of the *P53* gene has not been reported before. Considering the clinical data, abnormalities of the *P53* gene may have prognostic significance in acute leukemias, because four of the six patients with mutations died of aggressive disease. Although the frequency of the *P53* mutations detected in this study was low, there was high percentage of cases with increased mRNA level of *P53*. It indicates that in leukemias a mechanism other than mutation of the *P53* gene leads to its transcript accumulation and also further inactivation of P53 protein.

The amplification of *MDM2* gene was detected in only one sample from patient with ALL. This case revealed also *MDM2* and *P53* mRNA overexpression. The remaining cases with increased transcript level of *MDM2* demonstrated no *MDM2* gene amplification. These results suggest that mechanisms other than amplification of *MDM2* play a significant role in disordered *MDM2* mRNA expression, which may be one of many possibilities inactivating P53 suppressor function. A strong correlation between MDM2 protein overexpression and P53 protein overexpression in relapsed childhood ALL has been shown [37]. In this study a co-overexpression of *P53* mRNA

and *MDM2* mRNA was observed in 23% of ALL and in 19% of AML. These results suggest that overexpression of *MDM2* mRNA represents one of many alternative mechanisms of P53 inactivation in acute leukemias at diagnosis.

In conclusion, no statistically significant correlation between mRNA level of *P53* and *BCL2* and between mRNA level of *P53* and *BAX* was found (usually observed in normal condition), confirming that abnormalities of the P53 network are implicated in the pathogenesis of ALL and AML. The high frequency of *BCL2* mRNA overexpression and relatively low frequency of *BAX* mRNA overexpression detected in both analyzed leukemias, indicate that altered transcription of these genes may be involved in leukemogenesis.

## References


[1] ANAN K, MORISAKI T, KATANO M, IKUBO A, TSUKAHARA Y et al. Assessment of c-erbB2 and vascular endothelial growth factor mRNA expression in fine-needle aspirates from early breast carcinomas: pre-operative determination of malignant potential. Eur J Surg Oncol 1998; 24: 28–33.

[2] BATES S, VOUSDEN KH. P53 in signaling checkpoint arrest or apoptosis. Curr Opin Genet Dev 1996; 6: 12–18.

[3] BUESO-RAMOS CE, YANG Y, DELEON E, MCCOWN P, STASS SA et al. The human MDM-2 oncogene is overexpressed in leukemias. Blood 1993; 82: 2617–2623.

[4] CASTRO PD, LIANG JC, NAGARAJAN L. Deletions of chromosome 5q13,3 and 17p loci cooperate in myeloid neoplasmas. Blood 2000; 95: 2138–2143.

[5] CORDON-CARDO C, LATRES E, DROBNJAK M, OLIVIA MR, POLLACK D et al. Molecular abnormalities of mdm2 and p53 gene in adult soft tissue sarcomas. Cancer Res 1994; 54: 794–799.

[6] FINLAY CA. The mdm-2 oncogene can overcame wild-type p53 suppression of transformed cell growth. Mol Cell Biol 1993; 13: 301–306.

[7] GIROD SC, CESARZ D, FISHER U, KRUEGER GR. Detection of p53 and MDM2 protein expression in head and neck carcinogenesis. Anticancer Res 1995; 15: 1453–1457.

[8] GRAF J, MERK B, MAURER U, MULLER E, BERGMANN L. Identification of novel polymorphisms in intron 7 of the human p53 gene in acute myeloid leukemia and healthy donors. Leuk Lymphoma 2001; 41: 655–658.

[9] GREENBLATT MS. Mutation in the tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. Cancer Res 1994; 54: 4855–4878.

[10] HAINAUT P. The tumor suppressor protein p53 a receptor to genotoxic stress that controls cell growth and survival. Curr Opin Oncol 1995; 7: 76–82.

[11] HERMINE O, HAIOUN C, LEPAGE E, D'AGAY MF, BRIERE J et al. Prognostic significance of bcl-2 protein expression in aggressive non-Hodgkin's lymphoma. Blood 1996; 87: 265–172.

[12] HOLLSTEIN M, HERGENHAHN M, YANG Q, BARTSCH H, WANG ZQ et al. New approaches to understanding p53 gene tumor mutation spectra. Mutat Res 1999; 431: 199–209.

[13] HUNTER SB, ABBOT K, VARM VA, OLSON JJ, BARNET DW et al. Reliability of differential PCR for detection of EGFR and MDM2 gene amplification in DNA extracted from FFPE glioma tissue. J Neuropath Exp Neur 1995; 54: 57–64.

[14] KAWAMURA M, OHNISHI H, SHENG XM, MINEGISHI M, HANADA R et al. Alteration of p53, p21, p16, p15 and RAS gene in childchood T-cell acute lymphoblastic leukemia. Leukemia Res 1999; 23: 115–126.

[15] KORNBLAU SM, THALL PF, ESTROV Z, WALTERSCHEID M, PATEL S et al. The prognostic impact of BCL2 protein expression in acute myelogenous leukemia varies with cytogenetics. Clin Cancer Res 1999; 5: 1758–1766.

[16] KUSSIE PH, GORINA S, MARECHAL V, ELENBAAS B, MOREAU J et al. Crystal structure of MDM2 oncoprotein bound of the p53 tumor suppressor transactivation domain. Science 1996; 274: 948–953.

[17] LEE S, ELENBAAS B, LEVINE A, GRIFFITH J. P53 and its 14 KDa C-terminal domain recognize primary DNA damage in the form of insertion/deletion mismatches. Cell 1995; 81: 1013–1020.

[18] LEHMAN TA, HAFFTY BG, CARBONE CJ. Elevated frequency and functional activity of specyfic germline p53 intron mutation in familial breast cancer. Cancer Res 2000; 35: 1062–1069.

[19] MIYASHITA T, KRAJEWSKI S, KRAJEWSKA M. WANG HG, LIN HK et al. Tumor suppressor p53 is a regulator of Bcl-2 and bax gene expression in vitro and in vivo. Oncogene 1994; 9: 1799–1805.

[20] MOMAND J, JUNG D, WILCZYNSKI S, NILAND J. The MDM2 gene amplification database. Nucleic Acids Res 1998; 26: 3453–3459.

[21] MORI S, MURAKAMI-MORU K, JEWETT A, NAKAMURA S, BONAVIDA B. Resistance of AIDS-associated Kaposi's sarcoma cell to Fas-mediated apoptosis. Cancer Res 1996; 56: 1874–1879.

[22] NAKANO Y, NAOE T, KIYOI H, KITAMURA K, MINAMI S et al. Prognostic value of p53 gene mutations and the product expression in de novo acute myeloid leukemia. Eur J Haematol 2000; 65: 23–31.

[23] NOMDEDEU JF, LETE J, BAIGET M, LASA A, ESTIVILL C et al. Mutational analysis of P53 in 16 cases of acute lymphoblastic leukemia and Burkitt's lymphoma. Hematologica 1997; 82: 550–554.

[24] OHGAKI H, EIBL RH, SCHWAB M, REICHEL MB, MARIANI L et al. Mutations of the p53 tumor suppressor gene in neoplasma of the human nervous system. Mol Carcinogen 1993; 8: 74–80.

[25] OLTVAI ZN, MILLIMAN CL, KORSMEYER SJ. Bcl-2 heterodimers in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 1993; 74: 609–619.

[26] PROKOP A, WIEDER T, STURM I, EBMANN F, SEEGER K et al. Relapse in childhood acute lymphoblastic leukemia is associated with a decrease of the Bax/Bcl-2 ratio and loss of spontaneous caspase-3 processing in vivo. Leukemia 2000; 14: 1606–1613.

[27] REED JC. Bcl-2 family proteins. Oncogene 1998; 17: 3225–3236.

[28] ROBERTSON LE, PLUNKETT W, McCONNELL K, KEATING MJ, MCDONNELL TJ. Bcl-2 expression in chronic lymphocytic

leukemia and its correlation with the induction of apoptosis and clinical outcome. Leukemia 1996; 10: 456–459.

[29] SCHOTTELIUS A, BRENNSCHEIDT U, LUDWIG WD, MERTELSMANN RH, HERRMANN F et al. Mechanisms of p53 alteration in acute leukemias. Leukemia 1994; 8: 1673–1681.

[30] SHIEH SJ, MASAKO I, YOICHI T, PRIVES C. DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. Cell 1997; 91: 325–334.

[31] STIREWALT DC, KOPECKY KJ, MESHINCHIS S, APPELBAUM FR, SLOVAK ML et al. FLT3, RAS and TP53 mutation in elderly patients with acute myeloid leukemia. Blood 2001; 97: 3589–3595.

[32] STURM I, KOHNE CH, WOLFF G, PETROWSKY H, HILLEBRAND T et al. Analysis of the p53/BAX pathway in colorectal cancer: low BAX is a negative prognostic factor in patients with resected liver metastases. J Clin Oncol 1999; 17: 1364–1374.

[33] VOGELSTEIN B, LANE D, LEVINE AJ. Surfing the p53 network. Nature 2000; 408: 307–310.

[34] WADA M, BARTRAM CR, NAKAMURA H, HACHIYA M, CHEN DL et al. Analysis of p53 mutations in large series of lymphoid hematologic malignancies of childhood. Blood 1993; 82: 3163–3169.

[35] WANG TTY, PHANG JM. Effects of estrogen on apoptotic pathways in human breast cancer cell line MCF-7. Cancer Res 1995; 55: 2487–2489.

[36] WANNG Y, SZEKELY L, OKAN I, KLEIN G, WIMAN KG. Wild-type p53-triggered apoptosis is inhibited by Bcl-2 in a v-myc-induced T-cell lymphoma line. Oncogene 1993; 8: 3427–3431.

[37] ZHOU M, GU L, ABSHIRE TC, HOMANS A, BILLETT AL et al. Incidence and prognostic significance of MDM2 oncoprotein overexpression in relapsed childhood acute lymphoblastic leukemia. Leukemia 2000; 14: 61–67.

[38] ZHU YM, FORONI L, McQUAKER JG, PAPAIOANNOU M, HAYNES A et al. Mechanisms of relapse in acute leukaemia: involvement of p53 mutated subclones in disease progression in acute lymphoblastic leukaemia. Brit J Cancer 1999; 79: 1151–1157.