Altered expression of p53 and MDM2 proteins in hematological malignancies*

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In order to define the possible role of the MDM2 gene in the pathogenesis of human leukemia, the expression of MDM2 protein was examined in samples of fixed-permeabilized peripheral blood (PB) or bone marrow (BM) cells of leukemic patients by using flow cytometry. The present study showed, that normal PB and BM cells expressed low levels of MDM2. Overexpression of this protein was more frequently found in leukemic cells, namely in samples of patients with advanced, than those in incipient clinical stage of disease at examination. Of the 34 leukemias tested in our laboratory 24 (70%) showed abnormal expression of the MDM2 protein. This include 8/12 (66%) ALL, 10/13 (76%) B-CLL, and 6/9 (66%) AML. Since MDM2 and p53 are functionally related and overexpression of MDM2 can abrogate wild (wt)-p53 tumor suppressive function, we examined simultaneously with MDM2 protein expression also the expression of both wt-p53 and mutant (mt)-p53 with two MoAbs (Ab5 and Pab240). As measured by flow cytometry only a small part of the observed wt-p53 protein was in true wt-conformation (Ab5+), while most was in mt-conformation (Pab240+), which could mean, that most of the p53 protein in the cells was not functional, as in its usual role as a suppressor of the cell cycle. The MDM2 positive cases were negative for p53 (Pab240-) in hematopoietic cells of patients with B- and T-ALL at diagnosis and in relapsed disease. Samples of patients in remission with immunophenotype of normal cells were p53 and MDM2 negative. The expression of Ki67 antigen a nuclear protein associated with cell proliferation was used to verify the proliferative activity of the leukemic cells. Results of the two-color flow cytometric assay, which allows better definition of pathologic cell populations and nuclear fluorescence data for p53, MDM2 or Ki67 on a population of cells expressing only a given surface blast marker, confirmed their coexpression in the same cell.

Our preliminary results supported the view that the expression of p53 is very probably involved in the regulation of leukemic hematopoiesis and that the inhibition of p53 expression could modulate the proliferation of leukemic cells. It appears, that MDM2 overexpression, which may be p53-dependent, or also p53-independent plays an important role in leukemogenesis and/or disease progression.

Key words: p53, MDM2, flow cytometry, leukemia cells.

Inactivation by mutations is the most commonly detected abnormality in the p53 tumor suppressor gene. The MDM2 oncogene encodes a 90 kilodalton nuclear phosphoprotein that is induced by wild type (wt) p53 after DNA damage and inactivates p53 function, functioning as a p53 negative feedback regulator [1, 4, 8, 17]. Therefore, MDM2 may function both to enhance cell survival and to induce cell proliferation [22]. Although p53 alterations are common in human solid tumors, they are infrequent in hematological malignancies [19, 21]. Conversely, overexpression of MDM2 protein is frequently observed in hematological malignancies, particularly in patients with poor prognosis and advanced disease [3, 9, 10, 22, 26, 28, 29]. Importantly, MDM2 overexpression is not always related to alterations of p53, suggesting that MDM2 can impact on the growth and survival of tumor cells independent of p53 [15, 21, 23]. Although considerable attention has been focused on the structural changes in p53 and their biological consequences, the functional significance of MDM2 overexpression in human cancers is not well understood.

We have previously documented overexpression of p53 protein in some leukemia cells by flow cytometry [12, 13]. The present study may contribute to elucidation of some alternative mechanisms to p53 mutation for their functional

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inactivation. We examined p53 and MDM2 proteins simultaneously in the same sample of peripheral blood (PB) and/or bone marrow (BM) cells of patients with hematological malignancies, to asses their possible role in leukemogenesis.

Material and methods

Patients and cells. Peripheral blood (PB) and/or bone marrow (BM) samples from 14 healthy individuals which were observed for suspection of malignancy that was not proved and 35 patients (both children and adults) were used for detection of p53, MDM2 and Ki67 expression by flow cytometry. The mononuclear cell fraction was isolated by a standard density gradient centrifugation technique at a density of 1.077 g/ml. On the basis of immunological marker analysis of cells 10 patients were defined as early B-lymphoblastic leukemia (B-ALL), two as T-acute lymphoblastic leukemia (T-ALL), 14 patients were diagnosed as B-cell chronic lymphocytic leukemia (B-CLL) and 9 cases were classified as acute myeloid leukemia (AML).

Monoclonal antibodies (MoAbs). Immunophenotype analyses were carried out by flow cytometry using MoAbs against T-cell (CD1, CD2, CD3, CD4, CD5, CD7, CD8), B-cell (CD10, CD19, CD20, CD23) myeloid differentiation antigens (CD11b, CD13, CD14, CD15, CD33, CD65, MPO) and MoAbs against non-lineage restricted molecules (CD45, HLADR, CD34, CD38, CD71). MoAbs were purchased from Immunotech (Marseille, France) or Becton Dickinson (Mountain View, CA, USA). To estimate p53 protein expression two MoAbs were used: the antibody Ab5 (clone Pab1620) and Ab3 (clone Pab240). The first being purchased from Oncogene Research Products/Calbiochem (Cambridge, MA, USA) and the second one from Immunotech (Marseille, France). Anti-MDM2 (D-12) mouse monoclonal IgG1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and anti-Ki67 (Serotec, Oxford, UK) were also used. Mouse MoAbs of IgG1 or IgG2a isotypes, which were used as negative controls in the flow cytometric assay and fluorescein-isothiocyanate (FITC)- conjugated goat anti-mouse $F(ab)_2$ fragments as second antibody in indirect immunofluorescence assay with unlabeled MoAbs, were obtained from Immunotech (Marseille, France).

Surface and intracellular antigen immunostaining. The surface membrane markers on intact cells and nuclear/or cytoplasmic markers on fixed, permeabilized cells were determined by standard procedures for direct or indirect immunofluorescence assay [2]. When analyzed by flow cytometry the p53 protein keeps its native, non denatured form. Method of cell fixation by paraformaldehyde (PFA) (Sigma, St.Louis, USA) and permeabilization by methanol for their intracellular detection have been previously described in detail [12, 13]. Briefly, 1–2 x10^6 cells in 50 μl phosphate buffered saline (PBS) was mixed with 450 μl of 0.5% PFA solution in PBS and incubated at 37 °C for 10 min. Then the cells were cooled at 4 °C for 10 min and mixed with 500 μl of cold methanol and incubated at 4 °C for 30 min. The cells were washed twice with PBS. For the intracellular detection of MDM2 and Ki67 fixation/permeabilization method modified according to Drach at al [7] was used. It employs 1% PFA and 0.25% saponin (both Sigma, St. Louis, USA). The permeabilized cells were incubated for 15 min at room temperature with one of the mouse anti-human p53, anti-MDM2 or anti-Ki67 monoclonal antibodies or with a non-specific mouse isotype control. The antibody treated cells were washed twice with PBS and incubated with FITC-conjugated goat anti-mouse $F(ab)_2$ fragments for 15 min. The cells were then washed twice with PBS and analyzed using a flow cytometer. In dual-color experiments cells were first stained for membrane antigens using phycoerythrin (PE) labeled MoAbs before their fixation and permeabilization.

Flow cytometric analysis. Flow cytometric analysis was performed on a Epics Altra flow cytometer (Beckman Coulter, USA). At least 10 000 cells were analyzed in each sample. Results were expressed as percentage of positively labeled cells. Percentage of positivity was determined in the gate of pathologic population, or in remission samples in appropriate gates lymphoid or myeloid, respectively. Data were analyzed using WinMDI version 2.8 software (Scripps Research Institute, USA). Those cases with more than 20% reactive cells were considered to be positive for the respective antibody.

Results

PB and/or BM samples of normal and leukemic cells were studied for the expression of MDM2, p53 and Ki67 proteins. We developed a flow cytometric assay to evaluate the nuclear expression of MDM2 protein. Their relatively low levels in normal hematopoietic cells in peripheral blood and bone marrow are generally undetectable by flow cytometric analysis. Of the 34 leukemias tested in our laboratory by flow cytometry 24 (70%) showed abnormal accumulation of the MDM2 protein. These include 8/12 (66%) ALL, 10/13 (76%) CLL and 6/9 (71%) AML. Our preliminary results indicate, that overexpression of MDM2 protein was found significantly more frequently in patients with advanced than those in incipient clinical stage of disease at examination (Tab. 2). To asses the role of MDM2 protein in leukemogenesis, all cases were evaluated for MDM2 and p53 proteins expression simultaneously. Two monoclonal mouse anti-human antibodies were used in the flow cytometer analyses to detect p53 expression. The antibody Ab5 (clone Pab1620) recognizes only the wt-p53 protein, whereas Ab3 (clone Pab240) detects only mutated form of the p53 protein. The mutated p53 protein recognized by

Table 1. Flow cytometric expression of p53, MDM2 and Ki67 proteins in PB or BM cells of patients with early B-ALL at diagnosis, remission and relapse of disease and two patients with T-ALL at diagnosis

Patient No		Immunophenotype	Disease stage	Expression (%) Ab5	Pab240	MDM2	Ki67
B-ALL (early B-phenotype)							
1	PB	HLADR,CD19,CD34,CD38	diagnosis	3	2	60	55
2	BM	HLADR,CD19,CD10,CD34	diagnosis	3	2	65	20
3	PB	HLADR,CD19,CD10,CD34	diagnosis	3	16	49	nt
4	PB	HLADR,CD19,CD10,CD34	relapse	0	13	29	2
	BM	HLADR,CD19,CD10,CD34	relapse	0	5	57	25
5	BM	no blast markers	remission	0	1	4	0
6	BM	no blast markers	remission	0	4	7	0
7	BM	no blast markers	remission	0	2	0	0
8	BM	no blast markers	remission	0	3	5	3
9	BM	HLADR,CD19,CD10(63%)	after therapy	0	29	21	3
10	BM	HLADR,CD19,CD10(45%)	after therapy	0	2	38	3
T-ALL							
11	PB	CD10,CD4,CD8,CD7,CD3-	diagnosis	1	2	55	42
	BM	CD10,CD4,CD8,CD7,CD5,CD1,CD38,CD71,cCD3,TdT	diagnosis	2	1	56	46
12	PK	CD2,CD4,CD5,CD7,CD10	diagnosis	2	1	65	0
Controls mean of 14*			PB	0±0	2±1	2±1	0±0
			BM	0±0	4±2	2±2	1±1

nt – not tested, * – ±S.E.

Table 2. p53, MDM2 and Ki67 proteins expression in cells of B-CLL patients at different stages of disease at diagnosis and after therapy

Patient No		Immunophenotype	Disease stage	Expression (%) Ab5	Pab240	MDM2	Ki67
1	PB	HLADR,CD19,CD20,CD5	incipient	0	1	32	22
2	PB	HLADR,CD19,CD20,CD5,K	incipient	1	13	22	15
3	PB	HLADR,CD19,CD20,CD5,K	incipient	2	1	nt	nt
4	PB	HLADR,CD19,CD20,CD5	incipient	nt	nt	24	20
5	PB	HLADR,CD19,CD20,CD5	advanced	nt	nt	13	4
6	BM	HLADR,CD19,CD20,CD5	advanced	25	45	62	nt
7	PB	HLADR,CD19,CD20,CD5,K	advanced	9	62	64	nt
8	PB	HLADR,CD19,CD20,CD5	advanced	nt	nt	53	58
9	PB	HLADR,CD19,CD20,CD5,K	advanced	4	50	35	65
10	PB	HLADR,CD19,CD23,CD5	advanced	2	43	54	48
11	PB	HLADR,CD19,CD20,CD5	advanced	2	36	58	43
12	BM	of normal BM cells	after therapy	0	3	0	0
13	BM	of normal BM cells	after therapy	0	0	0	nt
14	PB	of normal PB cells	after therapy	1	1	28	3
Controls mean of 14*			PB	0±0	2±1	2±1	0±0
			BM	0±0	4±2	2±2	1±1

nt – not tested, * – ±S.E.

Ab3 can be either a protein translated from a mutated p53 gene or a wt-p53 protein that is only in mutational conformation. The proportions of positive cells in the samples are shown in Table 1, 2 and 3. The results from representative cases early B-, T-ALL, B-CLL, and AML are shown as histograms (Fig. 2a, c, 3a, 4a). In each of the samples p53 was expressed mostly in mutational conformation (Pab240 positive), whereas a negligible number of cells contained p53 in the wt conformation (Ab5 positive) (Tab. 1–3, Fig. 3a, 4a). MDM2 protein expression was studied and compared to p53 protein expression in the same samples of cells. The rate of MDM2 expression ranged from 21 to 67%. The MDM2 positive cases were negative for p53 (clone Pab240) in hematopoietic cells of patients with early B and T-ALL at

Table 3. p53, MDM2 and Ki67 expression in PB and/or BM cells of AML patients at diagnosis

Patient No		Immunophenotype	FAB subtype	Expression (%)			
				Ab5	Pab240	MDM2	Ki67
1	PB	HLADR,CD13,CD34	M1,M2	nt	nt	11	nt
2	PB	HLADR,CD13,CD34,cMPO	M1,M2	3	38	43	50
3	PB	CD33,CD11b,CD4,CD56,CD65	M2,M3	2	35	54	2
4	BM	HLADR,CD13,CD34,CD56	M2,M3	1	30	29	48
5	PB	CD13,CD33	M3	nt	nt	33	nt
6	PB	HLADR,CD33,CD14,CD11b	M4	4	34	43	26
	BM	HLADR,CD33,CD14,CD11b,CD38	M4	2	37	67	68
7	PB	HLADR,CD13,CD33,CD14,CD11b	M4	1	0	2	3
8	PB	HLADR,CD13,CD33,CD14,CD11b	M4	0	0	29	2
9	PB	HLADR,CD13,CD33,CD14,CD11b	M4	0	0	16	0
Controls mean of 14*			PB	0±0	2±1	2±1	0±0
			BM	0±0	4±2	2±2	1±1

nt – not tested, * – ±S.E., cMPO – cytoplasmic myeloperoxidase.

diagnosis and in relapsed disease (Tab. 1, Fig. 1a, c)) As it is shown in Table 1, Figure 1b, BM samples of patients in remission with immunophenotype of normal BM cells were p53 and MDM2 negative (patient No. 6–8). According to our results in two patients with early B-ALL after therapy (supposed to be in remission) with persistent cells with immunophenotype of cells of original pathological clone (HLADR+, CD19+, CD10+) in BM samples, p53 and MDM2 positivity (patient No. 9) or only MDM2 positivity (patient No. 10) was detected. The study included a group of 9 patients with newly diagnosed AML subdivided according to French-American-British (FAB) classification. Table 3 demonstrates, that 6 of 9 patients samples showed increased expression of p53 and MDM2 proteins in comparison to controls. The percentage of positive blasts did not significantly differ among the various FAB subtypes of AML.

The expression of Ki67 antigen, a nuclear protein associated with cell proliferation and found throughout cell cycle (G1-, S-, G2-, M-phases) was used in some cases to verify the proliferative activity of the leukemic cells. The percentage of Ki67 reactive cells ranged from 23 to 68% at diagnosis of ALL (2/5), AML (4/7) and only in advanced clinical stages of CLL (4/10) (Tab. 1–3). In some cases double staining was carried out with one of phycoerythrin-conjugated monoclonal antibody (CD7, CD19, CD33 or CD34) and fluorescein isothiocyanate-conjugated Pab240 or MDM2 or Ki67 to detect their coexpression in the same cell. Combined staining for intracellular MDM2 or Ki67 and membrane CD34 marker in PB cells of de novo diagnosed early B-ALL confirmed, that CD34+ cells were strongly positive for MDM2 (59%) and Ki67 (68%) expression in selected population of cells (Fig. 2b). The expression of MDM2 reactive PB cells of T-ALL patient was found to be closely associated with a population of CD7+ cells (65%) (Fig. 2d). Dual-color analysis by flow cytometry showed, that CD33 positive PB cells in AML patient (subtype M2, M3) were positive for Pab240 (25%) and MDM2 (50%) proteins (Fig. 4b). Double marker analysis in PB cells of B-CLL patient with advanced clinical stage of disease (Fig. 3b) showed that MDM2 and Ki67 proteins and membrane CD19 marker were mostly coexpressed in the same cells (63 to 82% in average).

Discussion

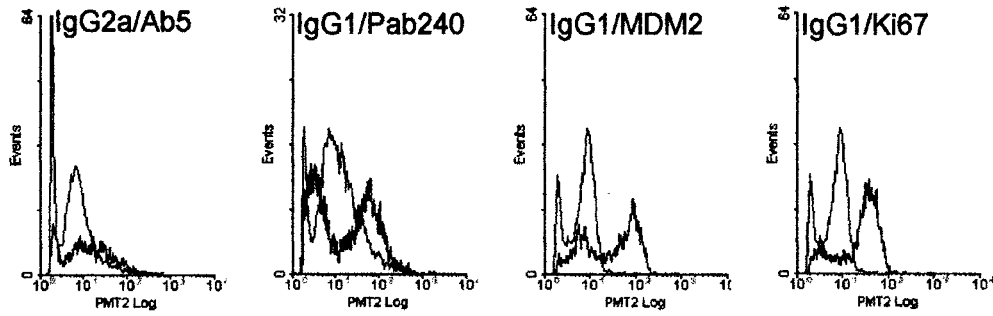
The MDM2 oncoprotein exists in an autoregulatory feedback loop with the tumor suppressor protein p53 [1, 8, 17]. Therefore, intracellular levels of these two proteins may play important role in cell proliferation and tumorigenesis. Although the mechanisms regulating MDM2 expression are not fully delineated, p53 is known to upregulate MDM2 expression. Previous reports [15, 21, 23] and also the results of the present study (Tab. 1, Fig. 1, 2) have shown, that overexpression of MDM2 protein in leukemic cells may not always correlate with status or expression of p53. This suggests, that MDM2 transcription may be regulated, at least in part, by other factors [24]. High levels of MDM2 and low levels of p53 protein support the view that overexpression of MDM2 may downregulate p53 protein expression. Since MDM2 can inhibit p53 mediated transactivation, MDM2 expression may be involved in the pathogenesis of some hematological malignancies, by deregulating the p53 dependent growth suppressive pathway [16, 20].

In order to define the possible role of the MDM2 gene in the pathogenesis of human leukemias, the intracellular expression of MDM2 protein was examined in samples of fixed-permeabilized PB/or BM cells of leukemic patients by using flow cytometry. The present study showed, that normal PB and BM cells expressed low levels of MDM2 (Tab. 1–3). Protein expression was more frequently over-

early B-ALL

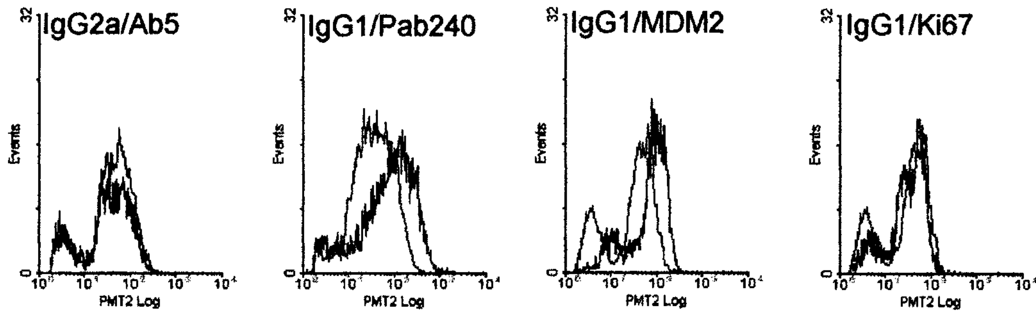
de novo diagnosis

a



remission

b



relapse

c

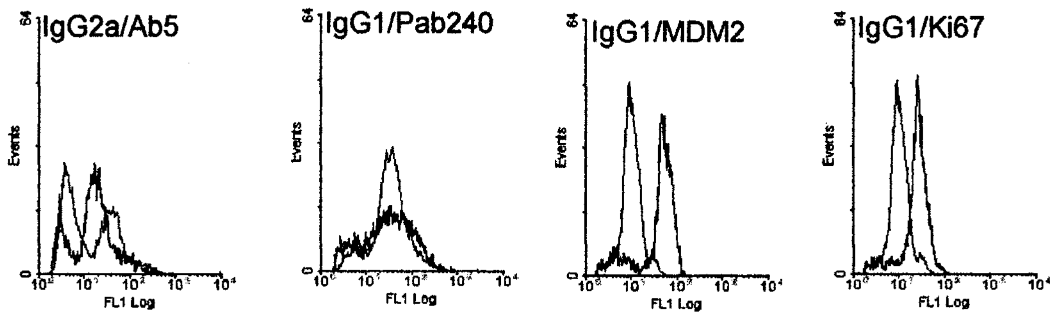
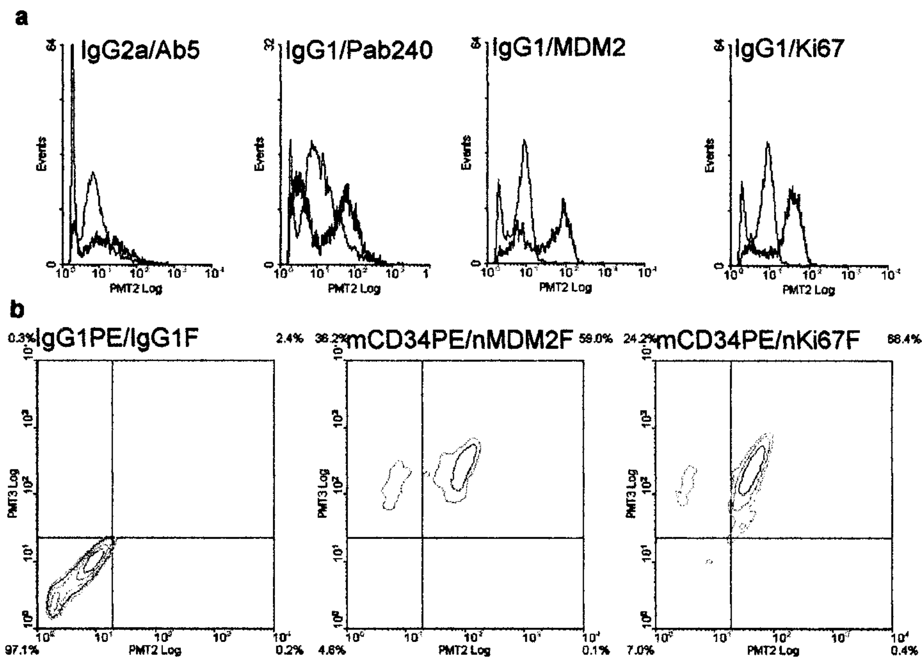


Figure 1. Comparative histograms of anti-Ab5 and IgG2a, anti-Pab240 and IgG1, anti-MDM2 and IgG1, anti-Ki67 and IgG1 MoAbs binding to intracellular structures of peripheral blood cells of early B-ALL patient at diagnosis of disease (a), at remission (b) and at relapse of disease (c). Abscissa – intensity of immunofluorescence, ordinate – number of cells.



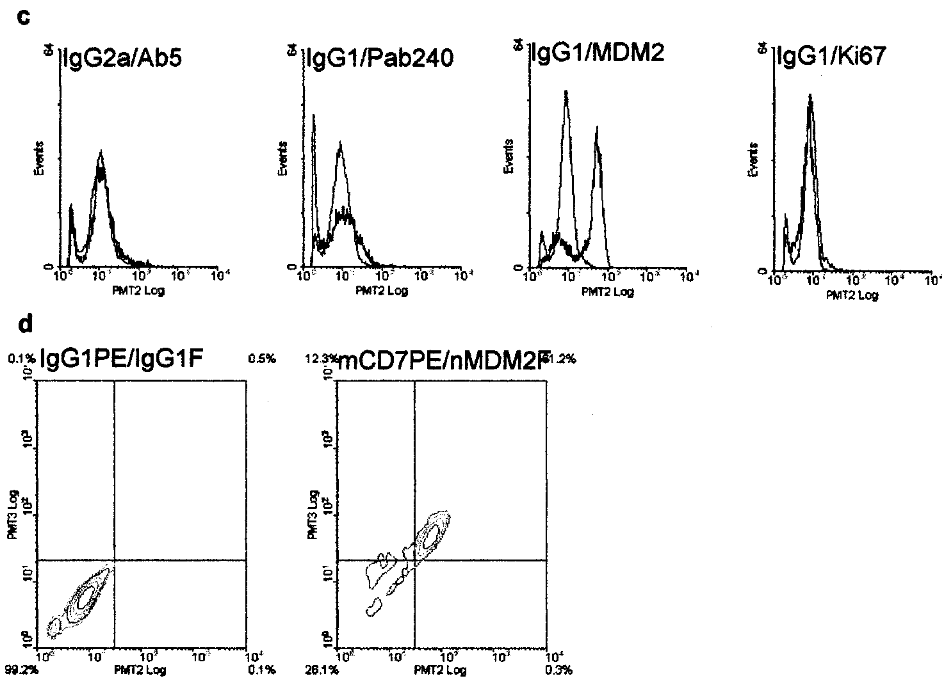


Figure 2. Comparative histograms of anti-Ab5 and IgG2a, anti-Pab240 and IgG1, anti-MDM2 and IgG1, anti-Ki67 and IgG1 MoAbs binding to intracellular structures of peripheral blood (PB) cells of early B-ALL patient at diagnosis of disease (a). Abscissa – intensity of immunofluorescence, ordinate – number of cells. Contour histograms of double staining on PB of early B-ALL patient at diagnosis (b). Negative isotypic control, membrane (m)CD34 and nuclear (n)MDM2 = 59%, mCD34 and nKi67 = 68%. Comparative histograms of anti-Ab5, and IgG2a, anti-Pab240 and IgG1, anti-MDM2 and IgG1, anti-Ki67 and IgG1 MoAbs binding to intracellular structures of PB cells of de novo diagnosed T-ALL patient (c). Abscissa – intensity of immunofluorescence, ordinate – number of cells. Contour histograms of double staining on PB cells of patient with T-ALL (d). Negative isotypic control, mCD7 and nMDM2 = 65%.

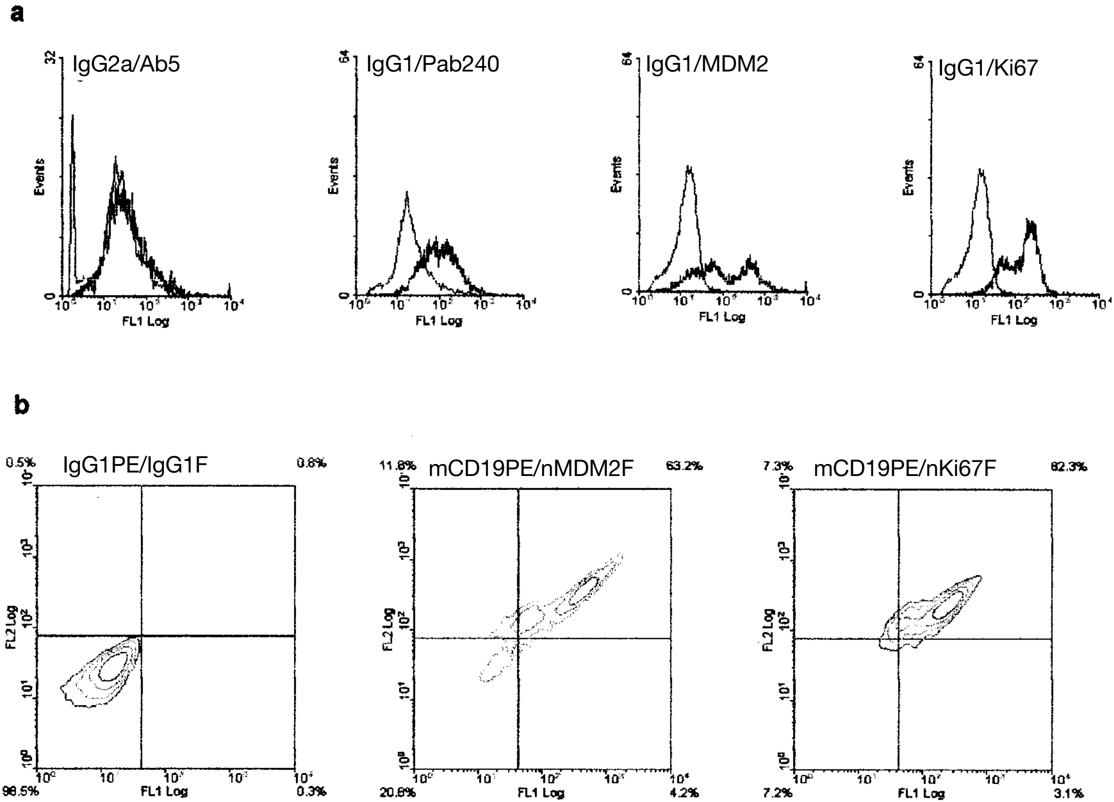


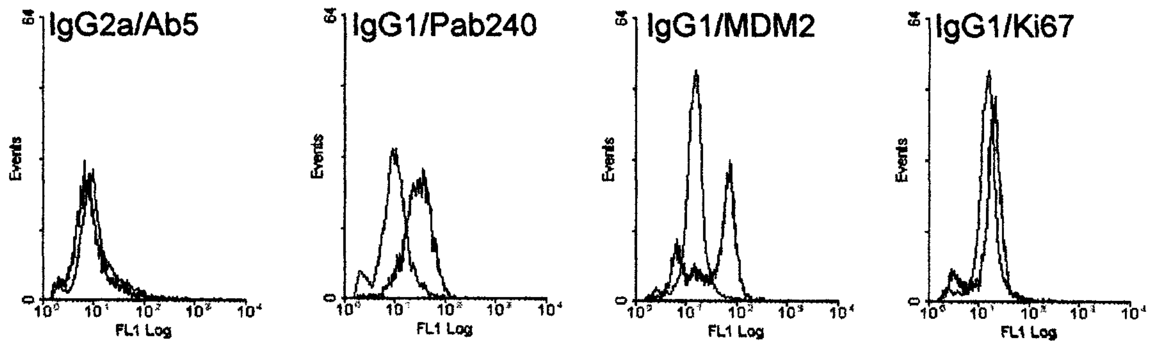
Figure 3. Comparative histograms of anti-Ab5, and IgG2a, anti-Pab240 and IgG1, anti-MDM2 and IgG1, anti-Ki67 and IgG1 MoAbs binding to intracellular structures of peripheral blood (PB) cells of B-CLL patient with advanced clinical stage of disease (a). Abscissa – intensity of immunofluorescence, ordinate – number of cells. Contour histograms of double staining on PB cells of the same B-CLL patient (b). Negative isotypic control of PB cells, membrane (m)CD19 and nuclear (n)MDM2 = 63% and mCD19 and nKi67 = 82%.

expressed in leukemic cells, namely in samples of patients with advanced than those in incipient clinical stage of disease at examination (Tab. 2), as well as p53 overexpressions reported in our previous reports [12, 13]. p53 and MDM2 presumably affect the same pathway. Thus we hypothesized that overexpressed MDM2 protein might bind to p53 and consequently play a role in the pathogenesis and or disease progression of patients who overexpress the MDM2 via inactivation of p53.

Since MDM2 and p53 are functionally related and overexpression of MDM2 can abrogate wt-p53 tumor suppressive function, we examined simultaneously the expression of both wt-p53 and mt-p53 with two MoAbs. Pab240 (Ab3) anti-p53 antibody binds to an epitope that is normally folded inside the p53 molecule where it cannot be accessed by the antibody [21, 25]. This antibody does not bind to wt-p53 in its native conformation and because most p53 mutations cause a change in conformation of the p53 molecule that exposes this epitope, thus antibody Pab240 binds to mutant p53. Antibody has been of particular interest as it has helped to define the occurrence of different conformational forms of the p53 protein [25]. It recognizes both mt-p53 and wt-protein in mutational, i.e. promoter but not in suppressor conformation and also reacts with denatured wt-protein. On the other hand, Ab5 antibody is known to recognize p53 only in wt- i.e. suppressor conformation, but not in promoter conformation [27]. It does not react with denatured or mutated protein either. The different reactivities with Ab5 and Pab240 in fixed-permeabilized cells may reflect genuine conformational differences of the p53 protein. Recent reports documented that the expression of a p53 protein reacting with Pab240 in hematopoietic cells is not always the result of mutations in the p53 gene [27], but the mutational p53 conformation has been also detected in normal hematopoietic progenitor cells [11, 18]. It is well known that wt-p53 protein may be present in two different conformations and their function in normal cell proliferation is conformation dependent [6]. It is believed that wt-p53 pro-

AML

a



b

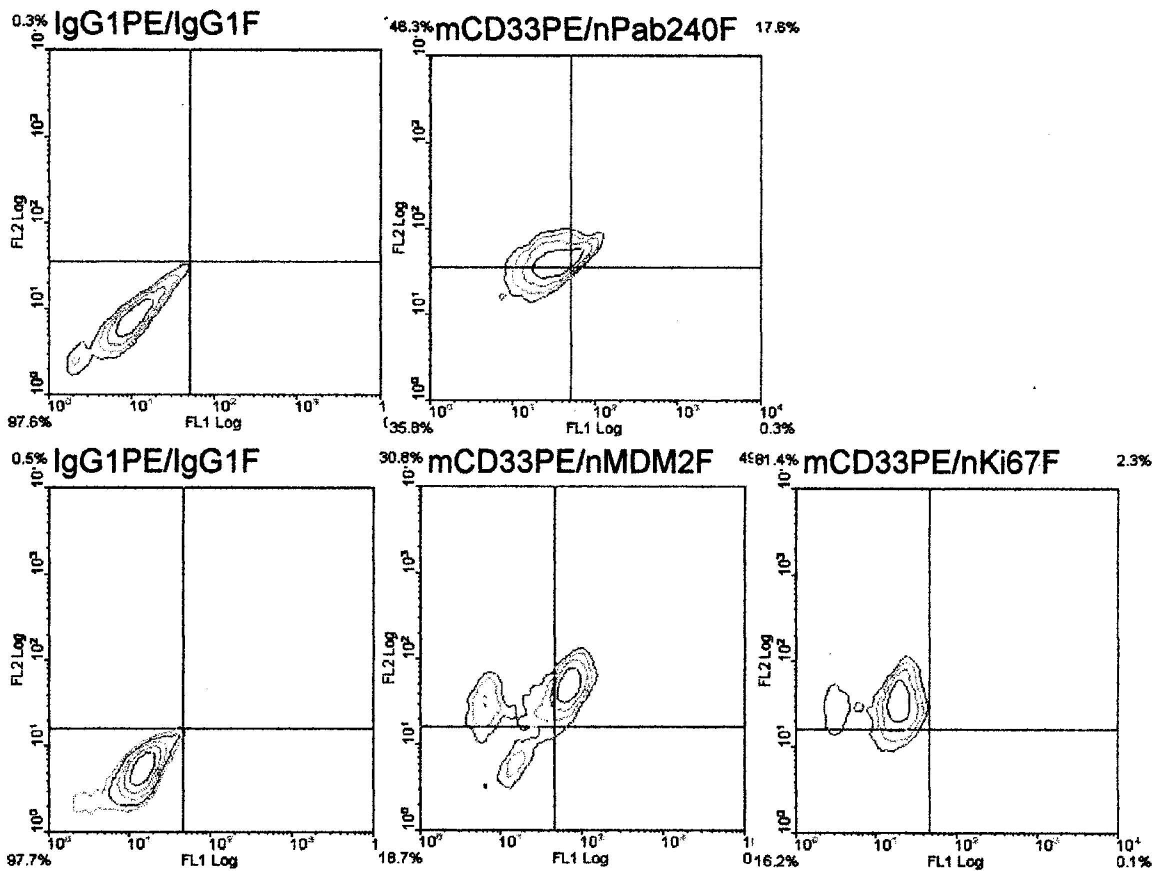


Figure 4. Comparative histograms of anti-Ab5 and IgG2a, anti-Pab240 and IgG1, anti-MDM2 and IgG1, anti-Ki67 and IgG1 MoAbs binding to intracellular structures of peripheral blood (PB) cells of AML patient (a). Abscissa – intensity of immunofluorescence, ordinate – number of cells. Contour histograms of double staining on PB cells of de novo diagnosed AML (M2, M3 subtype) patient (b). Negative isotypic control for nuclear (n)Pab240, membrane (m)CD33 and nPab240 = 25%, negative isotypic control for nMDM2 and nKi67 detection on PB cells, mCD33 and nMDM2 = 50% and mCD33 and nKi67 = 2%.

tein recognized by Pab240 may transiently lose its tumor suppressor and antiproliferative functions and thereby permit cell proliferation. Recently it was reported [27] that the p53 protein although not mutated, often adopted the conformation of mutant p53 as identified by antibody Pab240 in AML cells. This permanent alteration in conformation could be the mechanism causing their preferential proliferation over differentiation. Related or unrelated is the observation that about half of all AML samples have elevated expression of MDM2 protein when compared with normal cells. We reported increased expression of p53 (Pab240+) and MDM2 proteins in 5 of 9 patients at diagnosis of AML (Tab. 3). Examination of patients with B-CLL showed overexpression of MDM2 proteins in 10 of 13 cases, and in 5 of 13 cases p53 protein is overexpressed along with MDM2 (Tab. 2). In accordance with our results recent studies have suggested that MDM2 protein overexpression support its role of functional inhibitor of p53 and a potential poor prognostic factor for patients with CLL [10, 26]. p53 protein which was recognized by Pab240 antibody, was supposed to be in a mutational or promoter conformation. However, considering the oncogenic potential of overexpressed MDM2 protein, its possible role in the promotion of CLL disease remains to be evaluated. Overexpression of MDM2 has been demonstrated in 70% of leukemic patients tested in our laboratory by flow cytometry. In contrast, p53 expression was more heterogeneous than that of MDM2. The MDM2 positive cases were negative for p53 (clone Pab240) in hematopoietic cells of patients with early B- and T-ALL at diagnosis and relapsed disease (Tab. 1, Fig. 1a, c). The problem why p53 (Pab240+) overexpression was more frequently observed in B-CLL and AML than T-, or early B-ALL is unknown and further studies will be needed. The proliferative activity of cells was investigated in terms of Ki67 antigen detection, which has become a standard procedure for assessment of degree of cell proliferation in leukemic cells [5, 14, 20]. A detailed cell cycle analysis showed that Ki67 nuclear antigen is expressed in G1, S, G2 and mitosis, but not in G0. High expression of Ki67 in some leukemias could be associated with an overexpression of MDM2, which would act by a p53 independent mechanism. Results of the two-color flow cytometric assay, which allows better definition of pathologic cell populations and nuclear fluorescence data for p53, MDM2 or Ki67 present on a population of cells expressing only a given surface blast marker, confirmed their coexpression in the same cell (Fig. 2–4b). On the basis of these findings we conclude that flow cytometric evaluation of MDM2, Ki67 and p53 may be a useful tool for the study of the biological characteristics of the leukemic cells.

In conclusion, as measured by flow cytometry only a small part of the observed wt-p53 protein was in a true wt-conformation (Ab5+), while most part was in mt-conformation (Ab3-Pab240+). This finding could mean, that most of the p53 protein in the cells was not functional, in its usual role as a suppressor of the cell cycle. Our preliminary results showed overexpression of MDM2 protein in patients with some types of hematological malignancies. It appears, that MDM2 overexpression which may be p53-dependent or p53-independent could play an important role in leukemogenesis and-or in disease progression.

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References

[1] Alarcon-Vargas D, Ronai Z. p53-MDM2-the affair that never ends. Carcinogenesis 2002; 23: 541–547.

[2] Babušíková O, Glasová M, Koníková E, Kusenda J, Čáp J, Gyarfáš J, Koubek K. Phenotypic heterogeneity and aberrant markers expression in T-cell leukemia. Neoplasma 1998; 45: 128–133.

[3] Bueso-Ramos C, Manshouri T, Haidar MA, Huh YO, Keating MI, Albitar M. Multiple patterns of MDM-2 deregulation in human leukemias: Implications in leukemogenesis and prognosis. Leuk Lymphoma 1995; 17: 13–18.

[4] Capoulade C, Mir LM, Carlier K, Lécluse Y, Tétaud C, Mishal Z, Wiels J. Apoptosis of tumoral and nontumoral lymphoid cells is induced by both mdm2 and p53 antisense oligodeoxynucleotides. Blood 2001; 97: 1043–1049.

[5] Cordone I, Masi S, Mauro FR., Soddu S, Morsilli O, Valentini T, Vegna ML, Guglielmi C, Mancini F, Giuliacci S, Sacchi A, Mandeli F, Foa R. p53 expression in B-cell chronic lymphocytic leukemia: A marker of disease progression and poor prognosis. Blood 1998; 91: 4342–4349.

[6] Donehower LA, Bradley A. The tumor suppressor p53. Biochim Biophys Acta 1993; 1155: 181–205.

[7] Drach D, Drach J, Glassi H, Gattringer C, Huber H. Flow cytometric detection of cytoplasmic antigens in acute leukemias: Implications for lineage assignment. Leukemia Res 1993; 17: 455–461.

[8] Freedman DA, Levine AJ. Regulation of the p53 protein by the MDM2 oncoprotein-thirty-eight G.H.A. Clowes memorial award lecture. Cancer Res 1999; 59: 1–7.

[9] Gustafsson B, Christenson B, Gustafsson Be, Hjalmar V, Winiarski J. Cellular expression of MDM2 and p53 in childhood leukemias with poor prognosis. Med Ped Oncology 2000; 34: 117–124.

[10] Haidar MA, El-Hajj H, Bueso-Ramos CE, Manshouri T, Glassman A, Keating MJ, Maher A. Expression profile of MDM-2 proteins in chronic lymphocytic leukemia and their clinical relevance. Am J Hematol 1997; 54: 189–195.

[11] Kastan MB, Radin AI, Kuerbitz SJ, Onyekwere O, Wolkow CA, Civin CI, Stone KD, Woo T, Ravindranath Y, Craig

WR. Levels of p53 protein increase with maturation in human hematopoietic cells. Cancer Res 1991; 51: 4279–4286.

[12] Koníková E, Babušíková O, Kusenda J. Flow cytometry of p53 protein expression in some hematological malignancies. Neoplasma 1999; 46: 368–376.

[13] Koníková E, Kusenda J. p53 protein expression in human leukemia and lymphoma cells. Neoplasma 2001; 48: 290–298.

[14] Mansouri SE, Martin A, Mercadier A, Capoulade C, Maréchal V, Wiels J, Feuillard J, Rafaël M. High expression of MDM2 protein and low rate of p21waf1/CIP1 expression in SCID mice Epstein Barr Virus induced lymphoproliferation. J Histochem Cytochem 1999; 47: 1315–1321.

[15] Mosner J, Deppert W. p53 and MDM2 are expressed independently during cellular proliferation. Oncogene 1994; 9: 3321–3328.

[16] Pan Y, Haines DS. The pathway regulating MDM2 protein degradation can be altered in human leukemic cells. Cancer Res 1999; 59: 2064–2067.

[17] Prives C. Signaling to p53: Breaking the MDM2-p53 circuit. Cell 1998; 95: 5–8.

[18] Rivas CI, Wisniewski D, Strife A, Perez A, Lambek C, Bruno S, Darzynkiewicz ZC. Constitutive expression of p53 protein in enriched normal human marrow blast cell populations. Blood 1992; 79: 1982–1986.

[19] Schottelius A, Brennscheidt U, Ludwig WD, Mertelsmann RR, Herrman F, Lubbert M. Mechanism of p53 alteration in acute leukemias. Leukemia 1994; 8: 1673–1681.

[20] Stefanaki K, Tzardi M, Kouvidou CH, Chaniotis V, Bolioti M, Vlychou M, Zois M, Kakolyris S, Delides G, Rontogianni D, Georgoulias V, Kanavaros P. Expression of p53, p21, mdm2, Rb, bax and Ki67 proteins in lymphomas of the mucosa-associated lymphoid (MALT) tissue. Anticancer Res 1998; 18: 2403–2408.

[21] Takemoto S, Trovato R, Cereseto A, Nicot C, Kislyakova T, Casareto L, Waldmann T, Torelli G, Franchini G. p53 stabilization and functional impairment in the absence of genetic mutation or the alteration of the p14ARF-MDM2 loop in ex vivo and cultured adult T-cell leukemia/lymphoma cells. Blood 2000; 12: 3939–3944.

[22] Teoh G, Urashima M, Ogata A, Chauhan D, DeCaprio JA, Treon SP, Schlossman RL, Anderson KC. MDM2 protein overexpression promotes proliferation and survival of multiple melanoma cells. Blood 1997; 90: 1982–1992.

[23] Ungar S, Van de Maeren A, Tamilehto L, Linnainmaa K, Mattson K, Gerwin BI. High levels of MDM2 are not correlated with the presence of wild-type p53 in human malignant mesothelioma cell lines. Br J Cancer 1996; 74: 1534–1540.

[24] Urashima M, Teoh G, Chauhan D, Ogata A, Shirahama S, Kaihara C, Matsuzaki M, Matsushima H, Akiyama M, Yuza Y, Maekawa K, Anderson KC. MDM2 protein overexpression inhibits apoptosis of TF-1 granulocyte-macrophage colony-stimulating factor-dependent acute myeloblastic leukemia cells. Blood 1998; 92: 959–967.

[25] Vojtesek B, Dolezalova H, Lauerova L, Svitakova M, Havlis P, Kovarik J, Midgley CA, Lane DP. Conformational changes in p53 analyzed using new antibodies to the core DNA binding domain of the protein. Oncogene 1995; 10: 389–393.

[26] Watanabe T, Ichikawa A, Saito H, Hotta T. Overexpression of the MDM2 oncogene in leukemia and lymphoma. Leuk Lymphoma 1996; 21: 391–397.

[27] Zheng A, Castren K, Säilly M, Savolainen ER, Koistinen P, Vähäkangas K. p53 status of newly established acute myeloid leukemia cell lines. Brit J Cancer 1999; 79: 407–415.

[28] Zhou M, Heager AM, Smith SD, Findley HW. Overexpression of the MDM2 gene by childhood acute lymphoblastic leukemia cells expressing the wild-type p53 gene. Blood 1995; 85: 1608–1614.

[29] Zhou M, Gu L, Abshire TC, Homans A, Billett AL, Yeager AM, Findley HW. Incidence and prognostic significance of MDM2 oncoprotein overexpression in relapsed childhood acute lymphoblastic leukemia. Leukemia 2000; 14: 61–67.