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Human *Pim-2* Gene Expression in Acute Myeloid and Acute Lymphoblastic Leukemia Patients and Complete Remission

Ekspresja genu *hPIM-2* u pacjentów chorych na ostrą białaczkę szpikową i limfoblastyczną a remisja całkowita

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Abstract

Background. Human *Pim-2* is a proto-oncogene that encodes for serine/threonine kinase, which interacts with various signaling molecules. *hPim-2* is highly expressed in neoplastic tissues and in leukemic and lymphoma cell lines, which is consistent with its role during oncogenic transformation.
Objectives. The aim of this study was to investigate whether *hPim-2* expression is altered in acute myeloblastic leukemia (AML) and acute lymphoblastic leukemia (ALL).
Material and Methods. Thirty-seven patients were included: 22 with AML and 15 with B-cell ALL, aged 21–70 (mean: 41) years. Seventeen patients reached complete remission (CR). Bone marrow samples were collected at the time of diagnosis. Control samples were obtained from 8 healthy donors. *hPim-2* expression was analyzed by densitometric RT-PCR analysis.
Results. Expression of *hPim-2* in all leukemic patients (n = 37) and in both patient subgroups (AML and ALL) was significantly higher than in the controls ($p = 0.00001$, $p = 0.00009$, $p = 0.0002$, respectively). Patients who reached CR expressed *hPim-2* at significantly lower levels than those with primary resistance to chemotherapy (with no CR) ($p = 0.01$).
Conclusions. Moreover, correlation was found between *hPim-2* expression and patient age ($r = 0.45$; $p = 0.005$) and white blood cell count (WBC) ($r = 0.34$; $p = 0.04$). These data indicate that *hPim-2* gene expression is increased in patients with AML and ALL (**Adv Clin Exp Med 2010, 19, 1, 99–104**).

Key words: *hPim-2*, acute myeloid leukemia, acute lymphoblastic leukemia.

Streszczenie

Wprowadzenie. Protoonkogen *hPIM-2* koduje kinazę serynowo-treoninową, która odgrywa rolę w przenoszeniu różnych sygnałów. Wysoką ekspresję *hPIM-2* stwierdzono w komórkach nowotworowych oraz w białaczkowych i chłoniakowych liniach komórkowych, co wiąże się z jego rolą w procesie transformacji nowotworowej.
Cel pracy. Ocena ekspresji *hPIM-2* w komórkach białaczkowych u pacjentów chorych na ostrą białaczkę szpikową (OBS) i limfoblastyczną (OBL).
Materiał i metody. Do badania włączono 37 pacjentów; 22 z OBS i 15 z B komórkową OBL w wieku 21–70 lat (x = 41), 17 pacjentów osiągnęło remisję całkowitą (CR). Szpik kostny był pobierany od pacjentów w chwili rozpoznania. Grupę kontrolną stanowiło 8 zdrowych osób. Analizowano ekspresję *hPIM-2* za pomocą testu RT-PCR.
Wyniki. Średnia ekspresja *hPIM-2* była istotnie większa w stosunku do grupy kontrolnej we wszystkich badanych białaczkach, a także w podgrupach OBS i OBL (odpowiednio: p = 0,00001, p = 0,00009, p = 0,0002). U pacjentów, którzy uzyskali CR ekspresja ta była istotnie mniejsza niż u pacjentów bez CR (p = 0,01). Obserwowano ponadto, że ekspresja *hPIM-2* była istotnie większa w podgrupie pacjentów z OBS w stosunku do pacjentów z OBL (p = 0,04).
Wnioski. Dla całej populacji chorych na ostre białaczki stwierdzono statystycznie istotną dodatnią korelację między ekspresją *hPIM-2* a liczbą leukocytów (r = 0,34; p = 0,04) oraz wiekiem chorych (r = 0,45; p = 0,005). Badania własne wykazały, że ekspresja *hPIM-2* jest podwyższona u pacjentów z OBS i OBL (**Adv Clin Exp Med 2010, 19, 1, 99–104**).

Słowa kluczowe: *hPim-2*, ostra białaczka szpikowa, ostra białaczka limfoblastyczna.

Suppression of apoptosis is one of the most important phenomena inducing the accumulation of neoplastic cells in leukemia patients. Despite extensive research, the intracellular events leading to cell-life prolongation and resistance to pro-apoptotic factors are still not clearly determined. In recent years, the search for such events led to focusing on an anti-apoptotic factor, Pim-2. *Pim-2* was identified as an oncogene taking part in murine lymphoid cell transformation [1]. Pim-2, along with Pim-1 and Pim-3, belongs to the serine/threonine kinase family encoded by proto-oncogenes *hPim-1*, *hPim-2*, and *hPim-3* [2–4]. *hPim-2* gene expression is regulated both on the mRNA and protein level by numerous cytokines (especially IL-3) involved in the differentiation and maturation of hematopoietic cells [5, 6]. It has been detected in spleen, thymus, and proliferating hematopoietic cells [2]. Constitutive expression of hPim-2 extends cellular lifespan and makes the cells insensitive to pro-apoptotic stimuli, such as rapamycin, or deficiency of exogenous growth factors [5]. Animal model research showed that the Pim-1/Pim-2, like the independent anti-apoptotic PI3K/AKT/m-TOR pathway, are necessary for the normal differentiation, maturation, and survival of hematopoietic cells [7]. Double transgenic *Akt-1/Pim-2* mice developed lymphomas [7]. Similar observations were made by Allen et al., who revealed synergistic action of Pim-2 and c-Myc in lymphoma development in animals, and Zhang et al. in human cell lines [8, 9]. This indicates a possibility of this factor playing a role in neoplastic growth pathogenesis, which is confirmed by elevated expression of *hPim-2* in human primary solid tumor cell lines (G361, A549, SW480) as well as hematological cell lines (HL60, K562, RAIJ) [2]. Alterations in *hPim-2* gene expression regulation were also shown in cells derived from prostate cancer and in some lymphatic system neoplasms [10, 11]. In particular, *hPim-2* expression was increased in comparison to normal cells on both the mRNA and protein level in chronic lymphocytic leukemia and follicular and large-cell lymphoma. There was a connection between *hPim-2* level and disease stage and progression [11].

So far, no data regarding *Pim-2* mRNA and protein levels in acute leukemia are available. However, the observation that in some acute leukemia patients *hPIM-1* proto-oncogene is overexpressed suggests that disruption of the above pro-survival pathway may also occur in acute bone marrow neoplasms [12]. Therefore, the aim was to assess *hPim-2* gene expression in bone marrow samples collected from acute myeloid and lymphoblastic leukemia patients and to determine the correlation with clinical data and the outcome of induction treatment.

Material and Methods

Thirty-seven patients were included in the study, 22 with AML and 15 with B-cell ALL, aged 21–70 (median: 41) years. Of the AML patients, according to French-American-British (FAB) classification, 2 were suffering from M0 AML, 11 had M1, 4 had M2, and 5 had M4. All patients were treated at the Department of Haematology, Blood, Neoplasms and Bone Marrow Transplantation, Wroclaw Medical University. Hemoglobin concentrations ranged from 6.8–12.5 g/dl in the ALL group (mean: 9.5 ± 1.8 g/dl) and in the AML group 7.9–11.6 g/dl (mean: 9.6 ± 1.6 g/dl); white blood cell counts (WBC) from 1.4–360.5 × 10^9/l (mean: 76.3 ± 101.2 × 10^9/l) and from 2.8–285.0 × 10^9/l (mean: 48.02 ± 66.26 × 10^9/l), respectively. Blast cell counts in peripheral blood ranged from 0.9–301.4 × 10^9/l (mean: 62.2 ± 85.2 × 10^9/l) and from 1.1–250.8 × 10^9/l (mean: 39.6 ± 57.7 × 10^9/l), platelet counts from 1.0–318.0 × 10^9/l (mean: 56.5 ± 69.2) and from 2.0–255.0 × 10^9/l (mean: 53.8 ± 55.0 × 10^9/l). The content of blast cells in the myelogram ranged from 34–100% (mean: 79.9 ± ± 20.3%) and from 39–97% (mean: 74.1 ± 18.2%).

All of the patients underwent cytostatic treatment according to the PALG (Polish Acute Leukemia Group) program. Complete remission (CR) was obtained in 17 patients (11 with AML and 6 with ALL); the remaining 20 did not respond to the treatment. Patients were included in the study from January 1999 to July 2007 and were observed during periods of 6 to 108 months (mean: 34 months). The control group consisted of 8 hematologically healthy bone marrow donors matched by age and sex to the patients. The analyzed material was bone marrow obtained from the patients at diagnosis.

RT-PCR and densitometric analysis was applied to determine *hPim-2* expression. RNA was isolated from three million bone marrow mononuclear cells using a standard extraction method (TriPure Reagent, Roche Diagnostics). Complementary DNA (cDNA) was synthesized by AMV reverse transcriptase (Finnzyme). The primer sequences for *hPim-2* were forward: 5'-CTTTCCTTCCAATACCCCA-3' and reverse: 5'-CCATCTTCCATTCCTTCCC-3' (according to Cochen et al. [11]). A fragment of *c-ABL* gene, amplified simultaneously, was used as an internal control with the primers forward: 5'-CCCAACCTTTTCGTTGCACTGT-3' and reverse: 5'-TGA CTGGCGTGATGTAGTTGCTT-3'. The intensity

of each electrophoretic band was measured densitometrically in 2% agarose gel in the presence of ethidium bromide. Subsequently, the *hPIM2/c--ABL* ratio was calculated. Additionally, RNA isolated from the K562, HL60, and SD1 cell lines was analyzed.

Statistical analysis was performed using the Mann-Whitney *U* test for independent samples. Correlation between quantitative variables was tested with Spearman's rank correlation test.

Results

The mean expressions of *hPim-2* in all leukemic patients and in both the AML and the ALL subgroups were significantly higher than in the controls (p = 0.00001, p = 0.00009, and p = 0.0002, respectively). In the patients with CR, the expression was significantly lower than in those who did not respond to the induction treatment (p = 0.01). Moreover, significantly higher mean *hPim-2* expression was found in the AML than in the ALL subgroup (p = 0.04). The results are shown in Table 1 and Figs. 1 and 2.

For all leukemic patients, statistically significant positive correlations between *hPim-2* expression and WBC count (r = 0.34, p = 0.04) and patient age (r = 0.45, p = 0.005) were discovered. There was no relationship between *hPim-2* expression and absolute leukemic cell count in peripheral blood and bone marrow, hemoglobin concentration, platelet count, or the presence of chromosomal aberrations. *hPim-2* expression was additionally assessed in the K562, HL60, and SD1 cell lines. It was significantly higher than in the control group and comparable to the values obtained from all leukemic patients (p = 0.004, p = ns.).

Discussion

In recent years the interest of cancer research was focused on Pim-2 serine/threonine kinase. The growth factor-induced increase in Pim-2 expression (e.g. IL-3) suppresses apoptosis and promotes cell survival [2]. These events are a consequence of phosphorylation of pro-apoptotic factors, i.e. 4E-BP1 translation inhibitor and BAD protein, belonging to the BCL-2 family [13–15]. In particular, Pim-2 plays an important role in bone marrow cell growth, differentiation, and survival. Its action is convergent with another independent pro-survival pathway, PI3K/AKT/mTOR. Murine model analyses led to the observation that incapacity of one of these pathways may, at least partially, be compensated by the activity of the other [7].

The influence of Pim-2 activity on cellular proliferation is controversial. A study conducted on the pre-B-derived cell line FL5.12 indicated that *Pim-2* gene-transfected cells demonstrated longer survival despite the fact that over 99% were in G1 phase of the cell cycle [5]. Dai JM et al. observed that antisense oligonucleotides against *hPim-2* cause a significant decrease in the proliferating fraction of the DU-145 prostate cancer cell line [16]. High *hPim-2* expression (both on the mRNA and protein level) was also found by Gong et al. in human hepatocellular cancer cells (HepG2) and after *hPim-2* knock-down the cancer cells lost survival ability in IL-3 starvation medium [17]. Moreover, Zhang et al. presented specific and efficient silencing of hPim-2 gene expression by the siRNA (small-interfering RNA) method in the human colon cancer cell line SW480 [18]. The analyses of cell lines and a few observations made of lymphoma cells derived from patients indi-

Table 1. Mean *hPim-2* expression in healthy individuals and in AML/ALL patients

Tabela 1. Średnia ekspresja *hPim-2* u zdrowych osób i chorych na AML i ALL

	Control group (Grupa kontrolna)	AML + ALL patients	AML patients	AML + ALL CR	AML + ALL NCR	ALL	*p* value
	1	2	3	4	5	6	7
n	8	37	22	17	20	15	2 vs. 1 : 0.00001 5 vs. 4 : 0.01 3 vs. 1 : 0.00009 6 vs. 1 : 0.0002 3 vs. 6: 0.04
Mean (Średnia)	0.81	1.86	2.09	1.51	2.16	1.53	
SD	0.24	0.91	1.11	0.37	1.13	0.33	

N – number of patients; *SD* – standard deviation; AML – acute myeloid leukemia; ALL – acute lymphoblastic leukemia; CR – complete remission; NCR – no complete remission.

N – liczba pacjentów; *SD* – odchylenie standardowe; AML – ostra białaczka szpikowa; ALL – białaczka limfoblastyczna; CR – remisja całkowita; NCR – bez remisji.

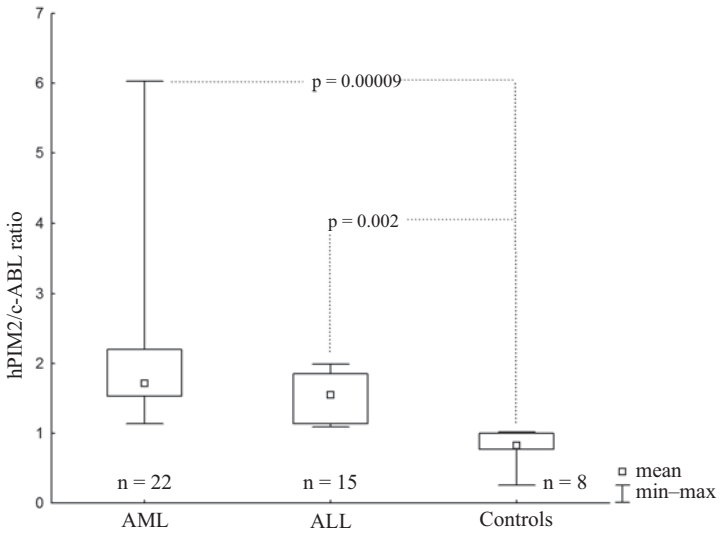

Fig. 1. Comparison of mean *hPIM-2* expression in patients with AML or ALL and the control group

Ryc. 1. Porównanie średniej ekspresji *hPIM-2* u pacjentów z AML i ALL oraz w grupie kontrolnej

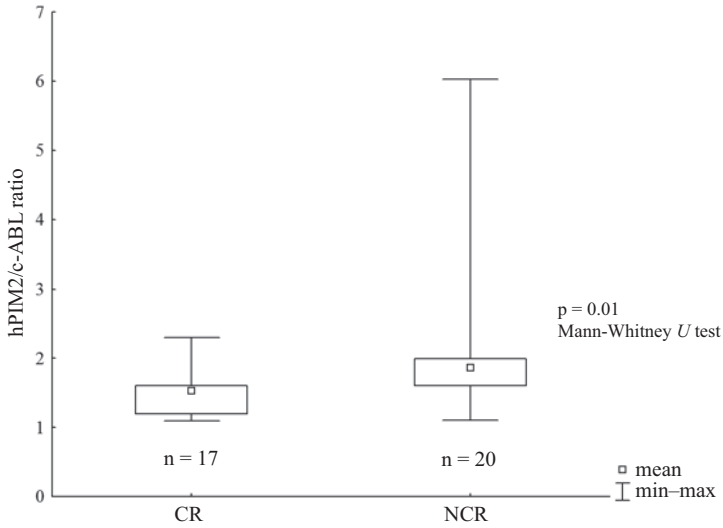

Fig. 2. Comparison of mean expression of *hPIM-2* in patients with and without CR

Ryc. 2. Porównanie średniej ekspresji *hPIM-2* u pacjentów z i bez CR

cate that increased hPim-2 expression may also be involved in the pathogenesis of bone marrow neoplasm. There are no publications regarding the role of this factor in acute leukemia development. The most recent research, conducted by Adam et al. on hematopoietic cells transformed by protein tyrosine kinases FTL3-ITD (Fms-like tyrosine kinase 3 internal tandem duplication) and BCR/ABL, frequently active in AML and ALL, demonstrated that the suppression of Pim-1 and Pim-2 expression a caused significant decrease in cell survival [19]. Therefore, the aim of the present study was to determine *hPim-2* expression levels in the bone marrow of AML and ALL patients and to compare them with those of a control group without pathological hematopoiesis.

It was found out that the content of *hPim-2* transcript was significantly higher in acute leukemia patients and in the HL60, K562 and SD1 cell lines than in the controls. These levels were also higher in the AML than in the ALL patients. The lack of a relationship between mRNA level and the percentage of leukemic cells suggests that increased expression of *hPim-2* gene occurs not only in blast cells, but also in morphologically normal hematopoietic cells. Aberrant regulation of expression might take place during the early stages of leukemogenesis. An important observation is that *hPim-2* expression was lower in the patients who reached complete remission compared with the group in which induction treatment was ineffective. This might support the thesis that hPim-2 promotes cell survival not only in leukemic, but also in normal hematopoiesis and decreases blast cell sensitivity to apoptosis, including that induced by cytotoxic drugs.

The assessment of correlations between *Pim-2* expression and activity and clinical features of the disease needs further evaluation. Consistent with the hypothesis of hPim-2's role in hematopoietic cell survival, this study demonstrated correlation between *hPim-2* mRNA level and WBC, but not with absolute blast cell count. The lack of a rela-

tionship between the expression of this gene and the number of peripheral blood blasts may result from the relatively small groups; however, the fact that such a correlation with the percentage of bone marrow blast cells was not observed is worth noting. As mentioned above, the possibility of increased *hPim-2* expression in leukemic and normal hematopoietic lines causing prolonged survival of both blast and mature cells cannot be excluded. These observations also indicate that there is a connection between *hPim-2* transcript levels in bone marrow cells and the outcome of induction treatment in acute leukemia. The correlation between this parameter and peripheral blood leukocyte count and patient age requires further elucidation of a potential dependence on other known prognostic factors.

The observed increase in *hPim-2* gene expression in the bone marrow cells of acute leukemia patients indicates its role in the pathogenesis of these diseases, especially those emerging from the myeloid line. This gene may also act protectively against pro-apoptotic cytostatic drugs. The present study should be regarded as preliminary and the significance of *hPim-2* gene expression as an independent prognostic factor regarding the outcome of induction treatment remains to be investigated further.

References

[1] **Breuer ML, Cuypers HT, Berns A:** Evidence for the involvement of pim-2, a new common proviral insertion site, in progression of lymphomas. EMBO 1989, 8, 743–748.

[2] **Baytel D, Shalom S, Madgar I, Weissenberg R, Don J:** The human Pim-2 proto-oncogene and its testicular expression. Biochim Biophys Acta 1998, 1442, 274–285.

[3] **Mikkers H, Allen J, Knipscheer P, Romeyn L, Hart A, Vink E, Berns A:** High-throughput retroviral tagging to indentify components of specific signaling pathways in cancer. Nat Genet 2002, 32, 153–159.

[4] **Reeves R, Spies GA, Kiefer M, Barr PJ, Power M:** Primary structure of putative human oncogene, pim-1. Gene 1990, 90, 303–307.

[5] **Fox JC, Hammerman PS, Cinalli RM, Master SR, Chodosh LA, Thompson CB:** The serine/threonine kinase Pim-2 is transcriptionally regulated apoptotic inhibitor. Gene Dev 2003, 17, 1841–1854.

[6] **Clemens MJ, Bushell M, Jeffrey I, Pain VM, Morley SJ:** Translation initiation factor modification and the regulation of protein synthesis in apoptotic cells. Cell Death Differ 2000, 7, 603–615.

[7] **Hammerman PS, Fox CJ, Brinbaum MJ, Thompson CB:** Pim and Akt oncogenes are independent regulators of haematopoietic cell growth and survival. Blood 2005, 105, 11, 4477–4483.

[8] **Allen J, Verhoeven E, Domen J, van der Valk M, Berns A:** Pim-2 transgene induces lymphoid tumors, exhibiting potent synergy with c-myc. Oncogene 1997, 15, 1133–1141.

[9] **Zhang Y, Wang Z, Li X, Magnuson NS:** Pim kinase dependent inhibition of c-Myc degradation. Oncogene 2008, 27, 4809–4819.

[10] **Dai H, Li R, Wheeler I, Diaz de Vivar A, Frolov A, Tahir S, Agoulnik I, Thompson T, Rowley D, Ayala G:** Pim-2 upregulation: biological implications associated with disease progression and perineural invasion in prostate cancer. Prostate 2005, 65, 3, 276–286.

[11] **Cohen AM, Grinblat B, Bessler H, Kristt D, Kremer A, Schwartz A, Halperin M, Shalom S, Merkel D, Don J:** Increased expression of the *hPim-2* gene in human chronic lymphocytic leukaemia and non-Hodgkin lymphoma. Leuk Lymphoma 2004, 45, 5, 951–955.

[12] **Amson R, Sigaux F, Przedborski S, Flandrin G, Givol D, Telerman A:** The human proto-oncogene product *p33pim* is expressed during fetal haematopoiesis and in diverse leukaemias. Proc Natl Acad Sci USA, 1989, 86, 8857–8861.

[13] **White E:** The pims and outs of survival signaling: role for Pim-2 protein kinase in the suppression of apoptosis by cytokines. Genes Dev 2003, 17, 1813–1815.

[14] **Yan B, Zemskova M, Holder S, Chin V, Kraft A, Koskinen PJ, Lilly M:** The PIM-2 kinase phosphorylates BAD on serine 112 and reverses BAD-induced cell death. J Biol Chem 2003, 14, 278, 46, 45358–45367.

[15] **Macdonald A, Campbell DG, Toth R, McLauchlan H, Hastie CJ, Arthur JSC:** Pim kinases phosphorylate multiple sites on Bad and promote 14-3-3 binding and dissociation from Bcl-XL. BMC Cell Biol 2006, 7, 1–14.

[16] **Dai JM, Zhang SQ, Zhang W, Lin RX, Ji ZZ, Wang SQ:** Antisense oligodeoxynucleotides targeting the serine/threonine kinase Pim-2 inhibited proliferation of DU-145 cells. Acta Pharmacol Sin 2005, 26, 3, 64–68.

[17] **Gong J, Wang J, Ren K, Liu C, Li B, Shi Y:** Serine/threonine kinase Pim-2 promotes liver tumorigenesis induction through mediating survival and preventing apoptosis of liver cell. J Surg Res 2008, 10.1016/j.jss.2008.03.033.

[18] **Zhang SQ, Du QY, Ying Y, Ji ZZ, Wang SQ:** Polymerase synthesis and potential interference of a small-interfering RNA targeting hPIM-2. World J Gastroenterol 2004, 10, 18, 2557–2660.

[19] **Adam M, Pogacic V, Bendit M, Chappuis R, Nawijn M,Duyster J, Fox CJ, Thompson CB, Cools J, Schwaller J:** Targeting PIM kinases impairs survival of haematopoietic cells transformed by kinase inhibitor-sensitive and kinase inhibitor-resistant forms of Fms-like tyrosine kinase 3 and BCR/ABL. Cancer Res 2006, 66, 7, 3828–3835.

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