THE *POU5F1* GENE EXPRESSION IN HUMAN LEUKEMIA CELLS

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S u m m a r y. Myeloid leukemia's are heterogenic group of neoplastic diseases with different course, treatment and prognosis. Among cancer cells isolated from the blood of patients suffering from leukemia, a small percentage constitutes CSC, or cancer stem cell with the properties of normal stem cells. Resistance of CSC to treatment, induction of recurrence and metastasis causes that more and more research groups carry out studies on these specific cells. OCT-3/4 is POU family transcriptional factor encoded by *POUF51*. OCT-3/4 plays a crucial role in the regulation and maintenance of stem cell pluripotency and the generation of induced pluripotent stem cells (iPS). In our study, the test group consisted of patients diagnosed with acute myeloid leukemia and chronic lymphocytic leukemia. We showed increased expression of *OCT-4* in all patient samples with the highest expression in patients suffering from Chronic Lymphatic Leukemia. AML with complex karyotype and translocation t(8;21) showed the highest expression in AML patients, then the lowest level was exhibited by cells in AML M2 and AML M4. We suggest that expression of *POUF51* depends on characteristics of clinically diagnosed leukemia, and coexisting translocations.
K e y w o r d s: OCT-3/4, POUF51, CSC, leukemia, AML, CLL.

INTRODUCTION

Myeloid leukemia belongs to neoplastic diseases with diversified illness trajectory. Cancer cells that stem from hematopoietic system occupy the peripheral blood, bone marrow and other body tissues. According to clinical cases, myeloid leukemia has been divided into two groups: acute and chronic leukemia. The exact causes of leukemia remain unclear [1]. Acute Myeloblastic leukemia (AML) is characterized by clonal proliferation and accumulation of morphologically and functionally immature blast cells that originate from precursor tumor-transformed myeloid cel [2]. AML is the most occuring acute leukemia in adults, with risk of development increasing with age [3].

Chronic lymphocytic leukemia (CLL) is monoclonal lymphoproliferative disease. Presented in the peripheral blood, bone marrow, lymph nodes and spleen, tumor cells come from morphologically mature but unfuncional B lymphocytes.[4] CML is identified by disease specific-immunophenotype: $CD19^+$, $CD20^+$, $CD23^+$and $CD5^+$ common for T lymphocytes [5]. CLL is the most common leukemia in adults, especially after the age of 60 [6].

During neoplastic investigation detected in solid tumor small amount (2%) cells which had properties of normal stem cells. They were called cancer stem cells. A lot of hypotheses have been put forward regarding the formation these cells. One of the most popular theories says, that cancer can arise from a normal tissue stem cell. Because they have a long time of life, they can accumulate a

lot of mutations. Cancer stem cells elude the control of proteins regulating the cell cycle, gaining "immortality". Strike to neoplastic transformation. Mutant CSCs produce a cell clone that not have the capacity of a stem cell. Other hypotheses suggest the possibility of CSC formation by mutation of progenitor cells or differentiated cells.

As a result a lot of changes in the genetic material of a cell, it may come to its "recession" in differentiation into the stem cell [7].

Due to the resistance of CSC to treatment, induction of recurrence and metastasis, more and more research groups take up the challenge of examine this cell group. Explanation of their formation and destruction in the human body, without damaging normal stem cells, gives hope for better treatment of cancer.

The search for early markers for leukemia is very important as they can be a useful diagnostic and prognostic instrument. Many studies showed, that the degree of differentiation of leukemia cells is correlated with prognosis. The more primitive cells, the worse prognosis. This allows us to speculate that markers associated with maintaining undifferentiated cell state may have prognostic meaning in the case of leukemias.

Oct-3/4 protein (*octamer-binding transcription factor-3/4*) encoded by the *POU5F1* gene (POU domain, class 5, transcription factor 1) located at locus 6p21.33, is a transcription factor belong to the POU family (Pit , Oct, Unc) [8]. Many studies showed that OCT-4 is expressed in immature or less differentiated cells [9]. POU5F1 is primaly expressed in embryonic stem cells (ESC) as it has crucial role in embrional development [10]. OCT-4 occurs in cancer stem cells and it has been showed that abnormal expression of *POU5F1* in somatic cells is associated with tumorogenesis. OCT3/4 plays a crucial role in the regulation and maintenance of stem cell pluripotency [11]. It is a pivotal transcriptional factor for obtaining of iPS (*induced pluripotent stem cells*) as it stands as one of four Yamanaka factors (Oct3/4, Sox2, Klf4, c-Myc) and its expression is essential for inducing pluripotency in both mouse and human somatic cells. Over-expression alone of OCT-4 can also generate iPS [12].

THE AIM OF THE STUDY

The aim of this study was to evaluate the expression of the *POU5F1* gene at the transcription level in acute myeloid leukemia cells and chronic lymphocytic leukemia.

MATERIALS

Tested group consisted of 20 patients of Clinic of Hematooncology and Bone Marrow Transplantation, Medical University of Lublin, including 11 patient diagnosed with chronic lymphocytic leukemia and 9 patients diagnosed with acute myeloid leukemia. Patients diagnosed with AML constituted heterogenic group: 2 of 9 patients suffering from AML with translocation t(8;21), 2 of 9 diagnosed with AML with complex karyotype, 2 of 9 with AML M2 (according to FAB classification), 1 of 9 with AML M4 (according to FAB classification), 1 of 9 with AML arising from an antecedent myelodysplastic syndrome and 1 of 9 with AML with features of differentiation.

As the material for the tests we used bone marrow collected from patients with AML and peripheral blood taken from patients with CLL.

Control group consisted of 16 patients, including 6 patients of Clinic of Trauma Surgery and Emergency Medicine, Medical University of Lublin from which normal bone marrow was taken during the hip endoprothesis replacements procedure, and 10 healthy volunteers from whom peripheral blood was collected.

The examinations were carried out with the consent of the Local Bioethical Commission.

METHODS

Examined material (peripheral blood and bone marrow) was harvested on EDTA and within 2 hours transported to laboratory, where mononuclear cells where isolated from blood and bone marrow by centrifuging in density gradient using reagent Histopague-1077 (Sigma) and PBS without Ca or Mg (Biomed). From acquired mononuclear cells of blood and marrow total cellular RNA was isolated, by modified Chomczyński method, using reagent TRI Reagent (Sigma), chloroform, isopropyl alcohol and 75% ethanol.

Isolated RNA was dissolved in ultrapure laboratory water then the purity and concentration of acquired extract was examined using Spectrofotometer (NanoDrop 2000c).

That RNA was used for reverse transcription reaction with a commercial reagent set High Capacity cDNA Reverse Transcription Kit with RNase inhibitors (Thermofisher Scientific).

POU5F1 gene expression was examined with qPCR method involving StepOnePlus system. For qPCR reaction TaqMqn type probes were used:

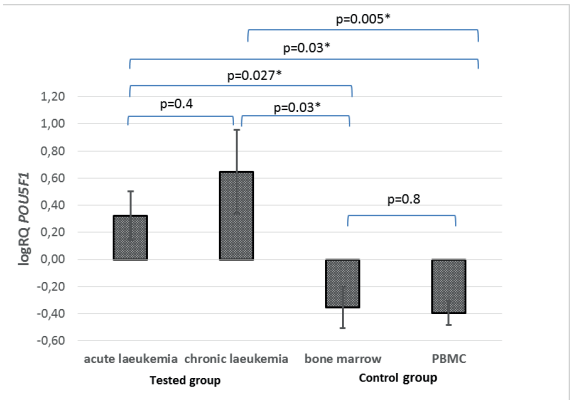
for examined gen *POU5F1* probe: Hs00999632_g1, for endogenic control *GAPDH* probe: Hs99999905_m1. The results of expression were analyzed with Expression Suite Software v1.0.3. Results of expression of examined gene were presented in a form of logarithmic relative value of the expression of studied gene (logRQ).

Statistic analysis was conducted with Statistica v13 software. For measuring differences between study groups ANOVA Kruskal-Wallis test was used (*p<0,05).

RESULTS

As the result of conducted test transcript of *POU5F1* gene was harvested from every examined cell. AML and CLL cell groups demonstrate significantly higher expression of *PUU5F1* in comparison to the normal peripheral blood and bone marrow cells (Figure 1). In leukemia's cells, the highest expression on *POU5F1* characterized cells harvested from patients suffering from Chronic Lymphatic Leukemia, nevertheless there was no significant difference in *POU5F1* expression between AML and CLL. Normal peripheral blood and bone marrow cells demonstrated importantly lower expression in comparison to leukemia cells, no difference in *POU51F* expression in normal blood cells and normal bone marrow cells was proved.

Separating cells harvested from patients with AML into subgroups shows following results: the highest *POU5F1* expression characterized AML with complex karyotype and AML with translocation t(8;21), whereas the lowest *POU5F1* expression was demonstrated by AML M4 according to FAB and AML M2 according to FAB (Figure 2).



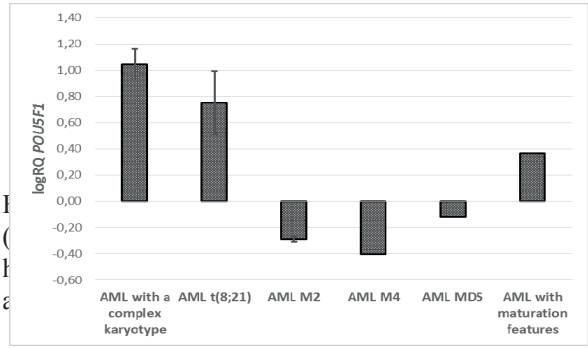


Figure 2. *POU5F1* gene expression (logRQ) in individual probes tested among bone marrow cells harvested from AML patients.

DISCUSSION

According to our research it has been shown that both blood and bone marrow cells of patients diagnosed with acute myeloid leukemia and chronic lymphocytic leukemia exhibit overexpression of *POU5F1* gene compared with normal blood and bone marrow cells. The current study demonstrates that hematological neoplasms are characterized by increased expression of *POU5F1* which is common for normal pluripotent stem cells. Further, attention was drawn to the fact that in AML patients group the highest expression of *POU51* showed cells with complex karyotype and translocation t(8;21). The lowest expression was found in AML M2 and AML M4 cells. These results indicate that expression of *POUF51* depends on characteristics of clinically diagnosed leukemia, coexisting translocations or the state of maturity of the tested cells.

Studies on *POU5F1* gene expression at transcription level in acute myeloid leukemia were also conducted by Picot et al. They showed increased *POU5F1* expression in leukemias cells compared to normal bone marrow cells. Our research corresponds to the results obtained by the Picot team, we also showed higher *POU5F1* gene expression at the transcription level in AML cells compared to normal marrow cells. In addition, the authors indicate that the less differentiated type of AML, characterized the higher *POU5F1* gene expression, our results also confirm this conclusion. It is speculated that higher expression of *POU5F1* gene in tumor cells can be responsible for maintaining the state of cell differentiation tumor stem cells. The prognostic expression of *POU5F1* gene in leukaemia has not been demonstrated so far [13].

In recent years various researches showed that presents of OCT-3/4 in cancer cells of pros-

tate, ovaries, cervix, urinary bladder, lungs, liver and pancreas. So far, there is not many reports on its role in blood cancers. In addition, other studies have shown that patients with tumors characterized by high expression of this gene have faster progression, more frequent metastases and greater mortality. Many reports suggest that OCT-4 is an unfavorable prognostic factor in solid tumors [14]. Kim et. al reported that OCT-4 overexpression in cervical cancer cells correlated with worse 5-year disease-free and overall survival rates when compared to low-expression group and OCT-4 was independent risk factor for overall survival [15]. Jing et al. associated level of OCT-4 with progression and prognosis in gastric cancer as it negatively correlated with 5 year survival rate in all stages of disease except for stage IV [16]. In hepatic cell carcinoma, Zhu et al. found increased level of OCT-4 in ZIC2-dependent mechanism as ZIC2 recruits NURF complex to bind to *OCT-4* promoter resulting in OCT-4 activation. They suggested that levels of ZIC2, NURF complex and OCT-4 can be used as diagnostic and prognostic markers in HCC patients [17]. Study of Hatefi et al. indicates that the level of *POU5F1* expression correlates with clinical and histopathological prognostic factor of bladder cancer and may potentially serve as prognostic marker of cancer [18]. Oct-3/4 may become as well a potential target for new cancer treatments. Samardzaja et al reported that in future, targeting of OCT4A and inhibition of its upstream targets such as WNT or AKT, may be a good strategy for preventing of tumor progression, subsequent recurrence and chemoresistnace in epithelial ovarian cancer [19, 21]. Further, Wang et al. demonstrated that OCT-4 may serve as new target for enhancing the chemosensitivity of cancers, as they examined that OCT-4 can mediate chemoresistance through OCT4-AKT-ABCG2 pathway [20].

CONCLUSIONS

Level of expression and clinical correlation of *POU5F1* expression still remain unclear in leukemia [11]. It is anticipated that in future expression of *POU5F1* may be use as important diagnostic molecular marker and therapeutic target for the development of new treatment strategies for leukemia [13]. Our research serve as assess the expression of this gene and its clinical relevance in patients with AML and CLL.

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