https://doi.org/10.15407/exp-oncology.2025.02.181

L. Shvachko [1], *, M. Zavelevich [2],
M. Dybkov [1], I. Gartovska [3], G. Telegeev [1]

[1] Institute of Molecular Biology and Genetics,
the NAS of Ukraine, Kyiv, Ukraine
[2] R.E. Kavetsky Institute of Experimental Pathology,
Oncology and Radiobiology, the NAS of Ukraine, Kyiv, Ukraine
[3] Kyiv Regional Oncology Center, Center for Hematology
and Bone Marrow Transplantation, Kyiv, Ukraine

* Correspondence: Email: l.shvachko@ukr.net

ALPHA-TOCOPHEROL AND G-CSF CHANGE EXPRESSION OF GENES ASSOCIATED WITH DIFFERENTIATION OF K562 CHRONIC MYELOID LEUKEMIA CELLS DOWNREGULATING EMT-ASSOCIATED STEMNESS BIOMARKERS

Background. Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder characterized by a block of myeloid differentiation, finally resulting in the uncontrolled expansion of CML stem cells in a phase of blast crisis of the disease. Tyrosine kinase inhibitors (TKI) are effective in delaying CML progression for a long time. Nevertheless, CML cells become resistant to TKI over time. Therefore, the search for alternative and complementary therapies, including differentiation therapy, is currently in the limelight. The **aim** of the study was to explore the differentiation potential of alpha-tocopherol and granulocyte-colony stimulating factor (G-CSF) by analyzing the gene expression of several factors critical for myeloid differentiation of K562 CML cells, as well as some key leukemic stemness transcription factors. **Materials and Methods.** The mRNA expression of C/EBPα (CCAAT/enhancer binding protein alpha), neutrophil-granulocytic factor TNAP (tissue non-specific alkaline phosphatase), E-cadherin, SNAIL, OCT4, and PLAP (placental-like alkaline phosphatase) was studied by qRT-PCR in K562 cells exposed to alpha-tocopherol or G-CSF. **Results.** K562 cell exposure to alpha-tocopherol or G-CSF resulted in the *CEBPB*, *CDH1*, and *ALPL* gene upregulation. At the same time, down-regulation of EMT-associated markers SNAIL, PLAP, and OCT4 (*SNAI1, ALPP,* and *POU5F1* genes) was demonstrated. **Conclusion.** The inverse relationship between expression of the genes of leukemic stemness cell markers SNAIL, OCT4, and PLAP and the genes of myeloid differentiation markers C/EBPα, TNAP, and E-cadherin in K562 cells exposed to alpha-tocopherol or G-CSF suggests the activation of the molecular pattern of myeloid differentiation in this setting.

Keywords: chronic myeloid leukemia, K562 cells, myeloid differentiation therapy, G-CSF, alpha-tocopherol.

Chronic myeloid leukemia (CML) is a clonal, hematopoietic stem cell (HSC) disorder characterized by a reciprocal translocation (t(9;22)(q34;q11) resulting in a fusion of the Abelson oncogene (*ABL*) located on chromosome 9q34 with the breakpoint cluster region (*BCR*) on chromosome 22q11.2 [1].

Citation: Shvachko L, Zavelevich M, Dybkov M, Gartovska I, Telegeev G. Alpha-tocopherol and G-CSF change expression of genes associated with differentiation of K562 chronic myeloid leukemia cells downregulating EMT-associated stemness biomarkers. *Exp Oncol.* 2025; 47(2): 181-187. https://doi.org/10.15407/exp-oncology.2025.02.181

The *BCR-ABL1* oncogene, product of the Philadelphia chromosome (Ph1) translocation, is the hallmark of chronic myelogenous leukemia (CML) [2, 3]. A fusion p210 BCR-ABL1 tyrosine kinase is an oncoprotein playing a pivotal role in CML pathology, diagnosis, and treatment as confirmed by the success of tyrosine kinase inhibitor (TKI) therapy [4, 5]. Imatinib, a BCR-ABL inhibitor, has emerged as the lead compound of the frontline therapy of CML [6, 7]. Meanwhile, despite advances in the development of more potent TKI, there are several factors leading to acquired therapy resistance, relapse, and finally — disease progression [8]. The maintenance of CML leukemic cell cells (LSCs) in patients who are resistant to TKI therapy indicates the existence of some mechanisms involved in the development of the resistance to therapy that are not completely dependent on BCR-ABL activity [9—12]. Therefore, the search for alternative and complementary therapies, including differentiation therapy, is currently in the limelight.

Over the past years, the inadequacy of many CML therapies has been shown to result from their failure to target LSCs. The control over the differentiation of CML LSCs may represent a possible therapeutic approach [13, 14].

The major molecular trigger of myelopoiesis is the myeloid master regulator transcription factor CCAAT/enhancer binding protein alpha (C/EBPα), which couples lineage commitment to the terminal neutrophil granulocytic differentiation and cell cycle arrest [15, 16]. However, immature CML blast cells lose their differentiation potential, acquiring an uncontrolled proliferation. The progression of CML to blast crisis is mainly correlated with down-regulation of C/EBPα [17]. In turn, the restoration of C/EBPα expression in the BCR-ABL+ cell line induces terminal granulocytic differentiation [18]. In CML, BCR-ABL protein inhibits C/EBPα translation via upregulation of poly(rC)-binding protein hnRNP-E2, and TKIs restore C/EBPα activity, facilitating granulocyte differentiation [19]. Therefore, C/EBPα may be suggested as a putative target in differentiation therapies of myeloid leukemias.

Earlier, we have demonstrated the ability of alpha-tocopherol to induce the restoration of C/EBPα expression, resulting in C/EBPα-mediated expression of the gene of G-CSF receptor (*CSF3R*) in the K562 CML cell line [20]. Meanwhile, G-CSF-R is expressed not only on such myeloid cells as neutrophils but also on HSCs and progenitor cells [21]. Taken together, these facts suggest a functional interrelationship between C/EBPα, G-CSF-R, and G-CSF [22, 23]. In fact, G-CSF is used in clinical practice for the mobilization of HSCs and progenitor cells in the setting of allogenic bone marrow transplantation [24].

Accumulating evidence shows the central role of G-CSF in the development of HSC progenitors in the common myeloid pathway in terminal neutrophil granulopoiesis [25]. Nevertheless, the possible involvement of G-CSF in the differentiation of CML blast cells has not yet been studied in detail. The loss of G-CSF and G-CSFR is a characteristic feature associated with the arrested differentiation of CML blast cells. G-CSF is currently used in CML patients, improving the responsiveness to TKI therapy. It is known that normal myelopoiesis in bone marrow is supported by G-CSF constitutively produced by bone-forming osteoblasts [26—28]. Meanwhile, tissue non-specific (liver/bone/kidney) alkaline phosphatase (TNAP), representing an osteogenic differentiation regulator of the osteoblasts, seems to be important in supporting the HSC niche in bone marrow [29]. G-CSF and TNAP can be suggested as mutually dependent markers of HSC differentiation. Moreover, TNAP encoded by the *ALPL* gene is detectable in the differentiated neutrophils and monocytes and induced by G-CSF treatment [30].

Previously, we have shown that in K562 CML cells, the *ALPL* gene was not expressed, while the expression of the aberrant isoform of alkaline phosphatase, namely leukemic stem cell-associated placental-like alkaline phosphatase (PLAP), was observed [31]. This aberrant isoform of alkaline phosphatase is considered potentially useful as a tumor-associated marker [32, 33]. Moreover, by our data, alpha-tocopherol is effective in restoring expression of C/EBPα and TNAP in K562 cells, both being tightly associated with possible reactivation of myeloid differentiation potential [31].

The aim of the present study was to further explore the differentiation potential of alpha-tocopherol and G-CSF by analyzing the expression of *CEBPB* and *ALPL* genes encoding for the factors critical for myeloid differentiation versus the expression of the genes encoding leukemic stemness-associated transcription factors SNAIL and OCT4 in K562 CML cells.

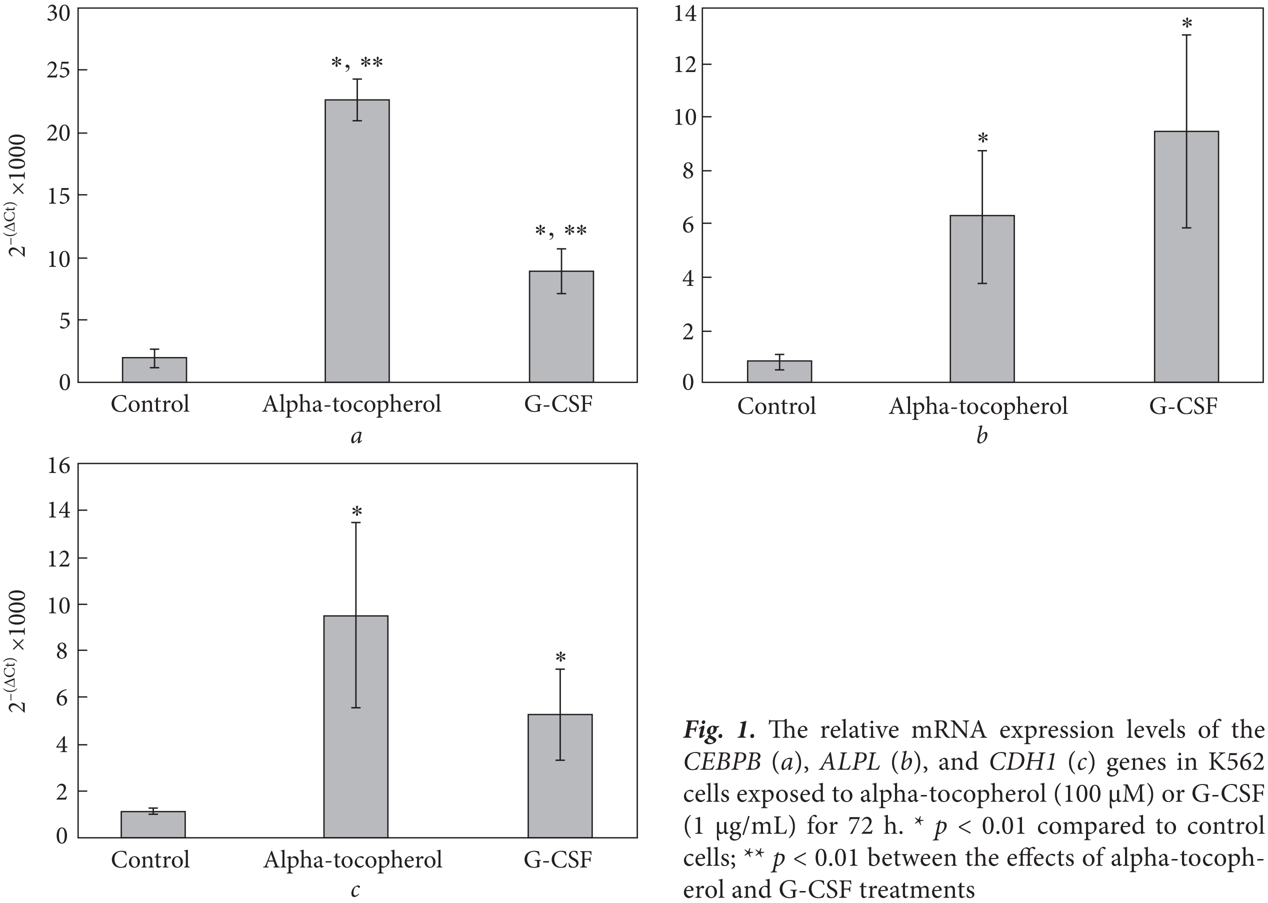


Fig. 1. The relative mRNA expression levels of the *CEBPB* (*a*), *ALPL* (*b*), and *CDH1* (*c*) genes in K562 cells exposed to alpha-tocopherol (100 μM) or G-CSF (1 μg/mL) for 72 h. * $p < 0.01$ compared to control cells; ** $p < 0.01$ between the effects of alpha-tocopherol and G-CSF treatments

Materials and Methods

The K562 cell line, originated from a CML patient in blast crisis, was obtained from the Bank of Cell Lines from Human and Animal Tissues of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, the NAS of Ukraine. K562 cells were grown in suspension in RPMI-1640 medium supplemented with 10% fetal calf serum. The cells were cultured at 37 °C in an atmosphere of 5% CO_2.

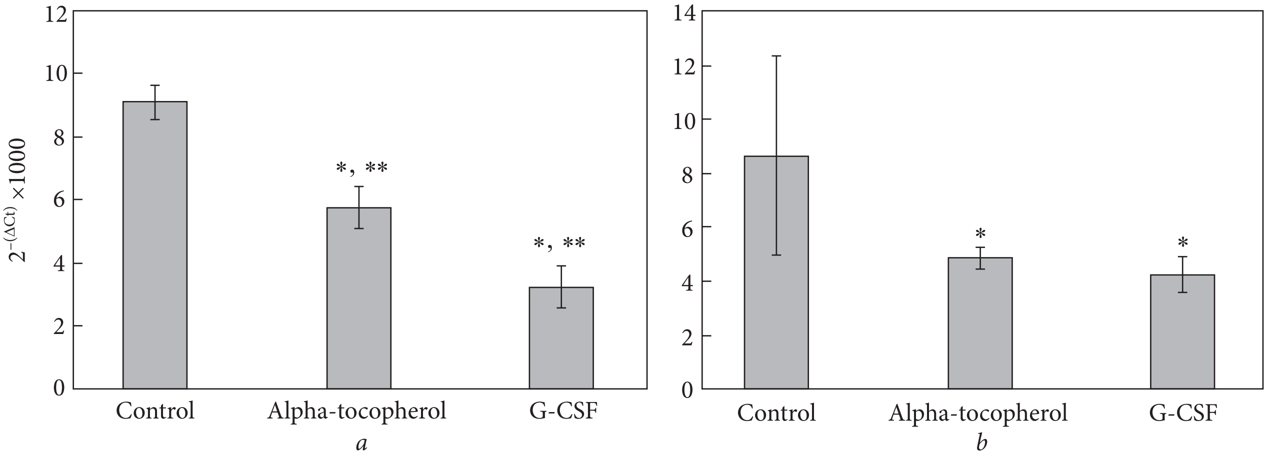
The cells were treated either with alpha-tocopherol acetate at a dose of 100 μM (300 mg/mL stock solution; Lekchim, Ukraine) or G-CSF (Filstim, Biofarma, Ukraine) at a dose of 1 μg/mL (10,000 IU). These optimal concentrations of both agents were determined in a preliminary set of experiments.

To dissolve the lipid-soluble alpha-tocopherol acetate, 30 μL of a stock solution of alpha-tocopherol was mixed with 10 μL DMSO with gently stirring, and 1 mL of water was added. This solution was further diluted 1:100 with culture medium. The cells were cultured with alpha-tocopherol acetate or G-CSF for 72 h.

Total RNA was extracted using TRIzol (Invitrogen, USA) according to the manufacturer's in-

Primers used in the study

Gene	Forward primer	Reverse primer
ALPL	5′-TGGCCGGAAATACATGTACCC-3′	5′-TTCCGTGCGGTTCCAGATG-3′
ALPP	5′-AAGGGCAGAAGAAGGACAAA	5′-GTCGTGTTGCACTGGTTAAAG
CEBPB	5′- CAA GAA CAG CAA CGA GTA CCG-3′	5′- GTC ACT GGT CAA CTC CAG CAC-3′
CDH1	5′-TGCCCAGAAAATGAAAAAGG-3′	5′-GTGTATGTGGCAATGCGTTC-3′
SNAI1	5′-CAGACCCACTCAGATGTCAA-3′	5′-CATAGTTAGTCACACCTCGT-3′
POU5F1	5′-GCACTGTACTCCTCGGTCCCTTTCCC-3′	5′-CTTCCCTCCAACCAGTTGCCCCAAAC-3′
GAPDH	5′- CGCTCTCTGCTCCTCCTGTT-3′	5′- CCATGGTGTCTGAGCGATGT-3′



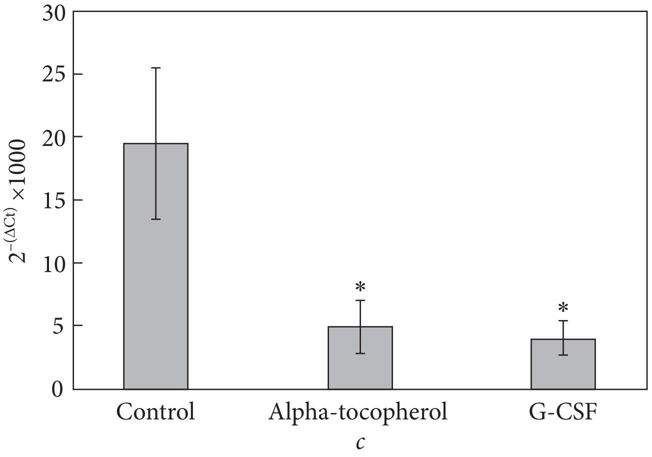


Fig. 2. The relative mRNA expression levels of *SNAI1* (*a*), *ALPP* (*b*), and *POU5F1* (*c*) in K562 cells exposed to alpha-tocopherol (100 μM) or G-CSF (1 μg/mL) for 72 h. * $p < 0.01$ (*a, c*) or $p < 0.05$ (*b*) compared to control cells; ** $p < 0.01$ between the effects of alpha-tocopherol and G-CSF treatments

structions. RNA was converted to cDNA using Qiagen's QuantiTect Rev. Transcription Kit (Qiagen, Germany). A real-time RT-PCR was performed using the SYBR Green protocol. The reaction mixture contained HotStarTaq DNA polymerase (Qiagen), 50 ng of cDNA, and SYBR Green at a 1:60,000 dilution. PCR conditions were: a 95 °C initial activation for 15 min was followed by 45 cycles of 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 30 s. The real-time RT-PCR was performed on a Bio-Rad Real-time RT-PCR Detection System IQ5 (USA). All the experiments were conducted in triplicate.

The primers used for the study (Table) were searched and designed by the Blast program (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and synthesized at the Institute of Molecular Biology and Genetics of the NAS of Ukraine.

The gene expression was quantified using the 2Δ-Ct method with normalization to *GAPDH* mRNA expression. The statistical significance of differences was evaluated by Student's *t*-test at the significance levels of $p < 0.05$ or $p < 0.01$.

Results and Discussion

First, we studied the effects of alpha-tocopherol and G-CSF on the expression of *CEBPB, ALPL,* and *CDH1* genes in K562 cells treated for 72 h (Fig. 1, *a*). Both agents upregulated *CEBPB* expression, but alpha-tocopherol caused 2.54 times higher upregulation of the gene compared to G-CSF. Both alpha-tocopherol and G-CSF upregulated *ALPL* expression at the same extent (Fig. 1, *b*). Coincidently with the expression of factors affecting differentiation of myeloid cells, we observed a significant increase in the expression of the *CDH1* gene (E-cadherin) in K562 cells treated with alpha-tocopherol or G-CSF (Fig. 1, *c*).

It is recognized that down-regulation of E-cadherin expression detected in various carcinomas correlates with the invasive-metastatic phenotype [34]. Although E-cadherin is not associated directly with differentiation of myeloid cells, the treatment of the K562 cells with such a differentiating agent as phorbol ether results in increasing expression of E-cadherin [35]. Moreover, E-cadherin is expressed on normal erythroid precursors and megakaryocytes but not on other hematopoietic marrow elements, while erythroleukemia cells lack E-cadherin expression [36].

The upregulation of the *CDH1* gene (E-cadherin), which coincided with the increasing expression of markers of myeloid differentiation such as C/EBP and TNAP induced by alpha-tocopherol or G-CSF prompted us to analyze the expression of several transcription factors that are upregulated in the setting of the loss of E-cadherin. It is known that the activation of such transcription factors as SNAIL, SLUG, TWIST1, ZEB1, ZEB2, and several others, which bind to E-box sequences in the *CDH1* gene promoter, negatively regulates E-cadherin expression [37, 38]. Moreover, these factors are crucial for maintaining cell pluripotency and stemness [39].

We found a significant down-regulation of *SNAI1, ALPP*, and *POU5F1* expression in K562 cells incubated with alpha-tocopherol (by 1.57, 1.78, and 3.93 times, respectively, compared to the control) or G-CSF (by 2.82, 2.05, and 4.83 times, respectively, compared to the control) (Fig. 2). Alpha-tocopherol was as effective as G-CSF in decreasing *ALPP* and *POU5F1* RNA expression, while its effect on the *SNAI1* expression was less noticeable as compared to G-CSF ($p < 0.01$) (Fig. 2).

While EMT has been implicated in promoting stemness of cancer cells, which is important for tumorigenesis and metastasis in solid tumors, the role of EMT and the major transcription factors involved in this process in the case of hematopoietic malignancies remains to be elucidated. Several studies assumed a role of such EMT-related factors as SNAIL, TWIST, and others in the pathogenesis of myeloid malignancies [40, 41]. In fact, we observed downregulation of SNAIL and OCT4 in CML K562 cells exposed to alpha-tocopherol or G-CSF.

Earlier, we have already found PLAP expression in peripheral blood cells of patients with CML in blast crisis, acute myeloid leukemia, and polycythemia vera, as well as in K562 cells [31]. In this study, we showed that PLAP downregulation is triggered not only by alpha-tocopherol but also by G-CSF as a specific inducer of myeloid differentiation. Moreover, the changes in the expression of PLAP caused by the differentiation agents are paralleled by the downregulation of the transcription factors SNAIL and OCT4 associated with the maintenance of the CSC stemness.

Taken together, the results obtained have emphasized that the detected in vitro downregulation of the stemness biomarkers SNAIL, PLAP, and OKT4 at the RNA level in K562 CML blast cells by alpha-tocopherol or G-CSF exposure is accompanied by the activation of the expression of the differentiation biomarkers E-cadherin, C/EBPα, and TNAP. This suggests a complementary approach to the CML therapy using the agents changing the balance between the transcription factors involved in cell differentiation, on the one hand, and cell stemness, on the other hand, as part of differentiation therapy of myeloid leukemias.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Acknowledgment

This work was supported by IMBG Simons Foundation Grant for Ukrainian institutions No. SFI-PD-Ukraine-00017453

REFERENCES

1. Shtivelman E, Lifshitz B, Gale RP, et al. Fused transcript of abl and bcr genes in chronic myelogenous leukaemia. *Nature*. 1985;315(6020):550-554. https://doi.org/10.1038/315550a0
2. Sayyler V, Griffin JD. Molecular mechanisms of transformation by the BCR-ABL oncogene. *Semin Hematol.* 2003;40:4-10. https://doi.org/10.1053/shem.2003.50034
3. Quintàs-Cardama A, Cortes J. Molecular biology of bcr-abl1-positive chronic myeloid leukemia. *Blood* 2009;113:1619-1630. https://doi.org/10.1182/blood-2008-03-144790
4. Pophali PA, Patnaik MM. The role of new tyrosine kinase inhibitors in chronic myeloid leukemia. *Cancer J.* 2016;22(1):40-50. https://doi.org/10.1097/PPO.0000000000000165
5. Jain P, Kantarjian H, Patel KP, et al. Impact of BCR-ABL transcript type on outcome in patients with chronic-phase CML treated with tyrosine kinase inhibitors. *Blood*. 2016;127(10):1269-1275. https://doi.org/10.1182/blood-2015-10-674242

6. Castagnetti F, Gugliotta G, Breccia M, et al. Long-term outcome of chronic myeloid leukemia patients treated frontline with imatinib. *Leukemia*. 2015;29(9):1823-1831. https://doi.org/10.1038/leu.2015.152
7. Gorre ME, Mohammed M, Ellwood K, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science*. 2001;293(5531):876-880. https://doi.org/10.1126/science.1062538
8. Jabbour E, Parikh SA, Kantarjian H, et al. Chronic myeloid leukemia: mechanisms of resistance and treatment. *Hematol Oncol Clin North Am*. 2011;25(5):981-995. https://doi.org/10.1016/j.hoc.2011.09.004
9. Houshmand M, Simonetti G, Circosta P, et al. Chronic myeloid leukemia stem cells. *Leukemia*. 2019;33:1543-1556. https://doi.org/10.1038/s41375-019-0490-0
10. Vetrie D, Helgason GV, Copland M. The leukaemia stem cell: similarities, differences and clinical prospects in CML and AML. *Nat Rev Cancer*. 2020;20(3):158-173. https://doi.org/10.1038/s41568-019-0230-9]
11. Xie X, Feng M, Wang Q, et al. Cellular and molecular state of myeloid leukemia stem cells. *Adv Exp Med Biol*. 2019;1143:41-57. https://doi.org/10.1007/978-981-13-7342-8_2
12. Hamilton A, Helgason GV, Schemionek M, Zhang B, Myssina S, Allan EK, et al. Chronic myeloid leukemia stem cells are not dependent on Bcr-Abl kinase activity for their survival. *Blood*. 2012;2119:1501-1510. https://doi.org/10.1182/blood-2010-12-326843
13. Chomel JC, Turhan AG. Chronic myeloid leukemia stem cells in the era of targeted therapies: Resistance, persistence and long-term dormancy. *Oncotarget*. 2011;2:713-727. https://doi.org/10.18632/oncotarget.333
14. Mojtahedi H, Yazdanpanah N, Rezaiel N. Chronic myeloid leukemia stem cells: targeting therapeutic implications. *Stem Cell Res Ther*. 2021;12:603. https://doi.org/10.1186/s13287-021-02659-1
15. Nelson DA. The biology of myelopoiesis. *Clin Lab Med*. 1990;10(4):649-659. PMID: 2272167
16. Porse BT, Bryder D, Theilgaard-Mönch K, et al. Loss of C/EBP alpha cell cycle control increases myeloid progenitor proliferation and transforms the neutrophil granulocyte lineage. *J Exp Med*. 2005;202(1):85-96. https://doi.org/10.1084/jem.20050067
17. Wagner K, Zhang Pu, Rosenbauer F, et al. Absence of the transcription factor CCAAT enhancer binding protein alpha results in loss of myeloid identity in bcr/abl-induced malignancy. *Proc Natl Acad Sci U S A*. 2006;103(16):6338-6343. https://doi.org/10.1073/pnas.0508143103
18. Tavor S, Park DJ, Gery S, et al. Restoration of C/EBP α expression in a BCR-ABL+ cell line induces terminal granulocytic differentiation. *J Biol Chem*. 2003;278(52):52651-52659. https://doi.org/10.1074/jbc.M307077200
19. Pulikkan JA, Tenen DG, Behre G. C/EBPα deregulation as a paradigm for leukemogenesis. *Leukemia*. 2017;31(11):2279-2285. https://doi.org/10.1038/leu.2017.229
20. Shvachko LP, Zavelevich MP, Gluzman DF, Telegeev GD. Vitamin E induces transcription factor C/EBP alpha and G-CSFR in K562 cells. *Exp Oncol.* 2018;40(4):328-331.
21. McKinstry WJ, Li CL, Rasko JE, et al. Cytokine receptor expression on hematopoietic stem and progenitor cells. *Blood*. 1997;89(1):65-71. PMID: 8978278
22. Semerad GL, Liu F, Gregory AD, et al. G-CSF is an essential regulator of neutrophil trafficking from the bone marrow to the blood. *Immunity.* 2002;17(4):413-423. https://doi.org/10.1016/s1074-7613(02)00424-7
23. Greenbaum AM, Link DC. Mechanisms of G-CSF-mediated hematopoietic stem and progenitor mobilization. *Leukemia*. 2011;25(2):211-217. https://doi.org/10.1038/leu.2010.248
24. Ji SQ, Chen HR, Wang HX, et al. Comparison of outcome of allogeneic bone marrow transplantation with and without granulocyte colony-stimulating factor (lenograstim) donor-marrow priming in patients with chronic myelogenous leukemia. *Biol Blood Marrow Transplant*. 2002;8(5):261-267. https://doi.org/10.1053/bbmt.2002.v8.pm12064363
25. Richards MK, Liu F, Iwasaki H, et al. Pivotal role of granulocyte colony-stimulating factor in the development of progenitors in the common myeloid pathway. *Blood*. 2003;102(10):3562-3568. https://doi.org/10.1182/blood-2003-02-0593
26. Calvi LM, Adams GB, Weibrecht KW, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature*. 2003;425:841-846. https://doi.org/10.1038/nature02040
27. Taichman RS, Emerson SG. Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor. *J Exp Med*. 1994;179:1677-1682. https://doi.org/10.1084/jem.179.5.1677
28. Fulzele K, Krause DS, Panaroni C, et al. Myelopoiesis is regulated by osteocytes through Gs -dependent signaling. *Blood*. 2013;121(6):930-939. https://doi.org/10.1182/blood-2012-06-437160
29. Nakamura T, Nakamura-Takashi A, Kasahara M, et al. Tissue-nonspecific alkaline phosphatase promotes the osteogenic differentiation of osteoprogenitor cells. *Biochem Biophys Res Commun*. 2020;524(3):702-709. https://doi.org/10.1016/j.bbrc.2020.01.136
30. Udristioiu A, Iliescu RG, Cojocaru M, Joanta A. Alkaline phosphatase isoenzymes and leukocyte alkaline phosphatase score in patients with acute and chronic disease: a brief review. *J Adv Med Med Res.* 2014;4(1):340-350. https://doi.org/10.9734/BJMMR/2014/3309
31. Shvachko LP, Zavelevich MP, Gluzman DF, Telegeev GD. Aberrant expression of placental-like alkaline phosphatase (PLAP) in chronic leukemia cells in vitro and its modulation by vitamin E. *Exp Oncol.* 2020;42(1):1-4. https://doi.org/10.32471/exp-oncology.2312-8852.vol-42-no-1.14285

32. Fishman WH. Clinical and biological significance of an isoenzyme tumor marker-PLAP. *Clin Biochem.* 1987;20:387-392.
33. Su Y, Zhang X, Bidlingmaier S, et al. ALPPL2 is a highly specific and targetable tumor cell surface antigen. *Cancer Res.* 2020;80(20):4552-4564. https://doi.org/10.1158/0008-5472.CAN-20-1418
34. Paćina-Slaus N. Tumor suppressor gene E-cadherin and its role in normal and malignant cells. *Cancer Cell Int.* 2003;3:17. https://doi.org/10.1186/1475-2867-3-17.
35. Zhou ZL, Ma J, Tong MH, et al. Nanomechanical measurement of adhesion and migration of leukemia cells with phorbol 12-myristate 13-acetate treatment. *Int J Nanomedicine.* 2016;11:6533-6545. https://doi.org/10.2147/IJN.S118065
36. Acs G, LiVolsi VA. Loss of membrane expression of E-cadherin in leukemic erythroblasts. *Arch Pathol Lab Med.* 2001;125(2):198-201. https://doi.org/10.5858/2001-125-0198-LOMEOE
37. Dongre A, Weinberg RA. New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. *Nat Rev Mol Cell Biol.* 2019;20:69-84. https://doi.org/10.1038/s41580-018-0080-4
38. Cano A, Pérez-Moreno MA, Rodrigo I, et al. The transcription factor SNAIL controls epithelial-mesenchymal transition by repressing E-cadherin expression. *Nat Cell Biol.* 2000;2(2):76-83. https://doi.org/10.1038/35000025
39. Chen SC, Liao TT, Yang MH. Emerging roles of epithelial-mesenchymal transition in hematological malignancies. *J Biomed Sci.* 2018;25:37. https://doi.org/10.1186/s12929-018-0440-6
40. Carmichael CL, Wang J, Nguyen T, et al. (2020). The EMT modulator SNAI1 contributes to AML pathogenesis via its interaction with LSD1. *Blood.* 2020;136(8):957-973. https://doi.org/10.1182/blood.2019002548
41. Dong CY, Liu XY, Wang N, et al. Twist-1, a novel regulator of hematopoietic stem cell self-renewal and myeloid lineage development. *Stem Cells.* 2014;32(12):3173-3182. https://doi.org/10.1002/stem.1803

Submitted: July 7, 2024

Л. Швачко [1], М. Завелевич [2], М. Дибков [1],
І. Гартовська [3], Г. Телегєєв [1]

[1] Інститут молекулярної біології і генетики НАН України, Київ, Україна
[2] Інститут експериментальної патології, онкології і радіобіології
ім. Р.Є. Кавецького НАН України, Київ, Україна
[3] Київський обласний онкологічний диспансер, центр гематології
і трансплантації кісткового мозку, Київ, Україна

АЛЬФА-ТОКОФЕРОЛ ТА Г-КСФ ЗМІНЮЮТЬ ЕКСПРЕСІЮ ГЕНІВ, АСОЦІЙОВАНИХ З ДИФЕРЕНЦІЮВАННЯМ КЛІТИН ХРОНІЧНОЇ МІЄЛОЇДНОЇ ЛЕЙКЕМІЇ К562, ЗНИЖУЮЧИ ЕКСПРЕСІЮ ЕМП-АСОЦІЙОВАНИХ БІОМАРКЕРІВ СТОВБУРОВОСТІ

Стан питання. Хронічна мієлоїдна лейкемія (ХМЛ) — клональний мієлопроліферативний розлад, який характеризується блокуванням мієлоїдного диференціювання, що призводить до неконтрольованої проліферації стовбурових клітин ХМЛ у фазі бластної кризи захворювання. Інгібітори тирозинкінази ефективні у відтермінуванні прогресування ХМЛ на певний час, однак згодом бластні клітини набувають резистентності до їхньої дії. Таким чином, пошук альтернативних та додаткових засобів впливу, включаючи і диференціювальну терапію, є актуальним. **Мета** роботи полягала у виявленні диференціювального потенціалу альфа-токоферолу та гранулоцитарного колонієстимулювального фактора (Г-КСФ) шляхом аналізу експресії генів декількох факторів, критичних для мієлоїдного диференціювання клітин К562, а також ключових транскрипційних факторів, що визначають стан стовбуровості клітин. **Матеріали та методи.** Експресію мРНК C/EBPα (CCAAT/enhancer binding protein alpha), нейтрофільно-гранулоцитарного фактора TNAP (тканинна неспецифічна лужна фосфатаза), Е-кадгерину, SNAIL, OCT4 та PLAP (плацентарно-подібної лужної фосфатази) визначали методом кількісної ЗТ-ПЛР у клітинах К562, інкубованих у присутності альфа-токоферолу або Г-КСФ. **Результати.** Інкубація клітин К562 з альфа-токоферолом або Г-КСФ приводить до підвищення експресії генів *CEBPB*, *CDH1* та *ALPL* та зниження експресії генів *SNAI1, ALPP* та *POU5F1*, які кодують відповідно SNAIL, PLAP та OCT4, що асоціюються з ЕМП. **Висновок.** Виявлена обернена залежність між експресією генів маркерів стовбуровості лейкемічних стовбурових клітин (SNAIL, OCT4, PLAP) та генів маркерів мієлоїдного диференціювання (C/EBPα, TNAP, Е-кадгерин) у клітинах К562 за дії альфа-токоферолу або Г-КСФ дозволяє припустити активацію молекулярного патерну мієлоїдного диференціювання в цих клітинах.

Ключові слова: хронічна мієлоїдна лейкемія, клітини К562, диференціювальна терапія, Г-КСФ, альфа-токоферол.