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VITAMIN E VERSUS IMATINIB TARGETS SNAIL -EMT PHENOTYPE IN CML BLAST CRISIS LEUKEMIC K562 CELLS IN VITRO

L. P. Shvachko*

Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine.

***Corresponding Author: L. P. Shvachko**

Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine.

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ABSTRACT

Background.The BCR-ABL- positive chronic myeloid leukemia (CML) is leukemic stem cells (LSCs) disease undergoing EMT stemness phenotype development. Therefore, CML progression results in EMT stemnes -LSCs-derived undifferentiated blast cells crisis (BC-CML). Imatinib targeting CML marker p210BCR-ABL oncogene tyrosinekinase and acting as an ATP-competitive inhibitor of the ABL1 kinase domain recently is successfull in TKI- therapies with a long enough CML remission. But imatinib as well as a TKIs still leads to EMT stemness-derived resistance. The Aim is search for alternative mechanism in overcoming EMT-stemnes LSCs phenotype in CML blast crisis progression by Vitamin E in versus imatinib. The Methods. The CMLblast crisis leukemic K562 cells exposed by vitamin E (100 μM) and Imatinib (Glivek) (2,5 mM) under 48 h were studied on the mRNA gene expression level of transcription factor SNAIL, as key stemnes EMT-inductor and C/EBPα transcription factor as pivotal master regulator of myelopoiesis by real-time qRT-PCR assay. The Conclusion. On the date obtained we have elucidated the principal different mechanisms of relative increasing of C/EBPα mRNA expression in CML blast crisis K562 cells exposured by vitamin E and Imatinib (Glivek). The mechanism of action of vitamin E is associated with the reprogramming of the EMT-SNAIL phenotype in CML blast cells to differentiation potential while the action of Imatinib focuses only on the inhibition of BCR-ABL -tyrosine kinase in CML blast cells that resulting to derepression of the main target CEBPα myeloid differentiation factor. The obtained results can significantly supplement the mechanism of acquisition of Imatinib resistance through activation of SNAIL-EMT-leukemic stem cell phenotype in CML progression.

KEYWORDS: chronic myeloid leukemia, imatinib-resistance, Snail - EMT stemness, vitamin E, myeloid master regulator C/EBP α.

INTRODUCTION

Chronic myeloid leukemia (CML) is a type of hematological cancer that starts in the myeloid progenitor blood-forming cells of the bone marrow and invades the blood as immature blast cells.[1] Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder associated with the activity of BCR-ABL fusion oncogene due to the reciprocal translocation t(9;22)(q34;q11).[2,3] BCR-ABL is the driving force of leukemogenesis in CML. The BCR/ABL fusion protein with elevated ABL tyrosine kinase activity is crucial for transformation of hematopoietic cell.[4] Tyrosine kinase inhibitors (TKIs), as imatinib, nilotinib, dasatinib, bosutinib, are the current goal treatment of CML.[5] This "Initial" treatment is the first therapy given for a disease. In most patients, TKIs can control CML. Despite a major clinical advance in the treatment of CML, TKI (Imatinib) resistance has become a challenging problem. It is known BCR-ABL point mutations are the principal cause of resistance to the treatment resulting in CML relapse and progression.[6,7] But the persistence of leukemic stem cells (LSCs) remains a major obstacle to cure CML.[8, 9] It is recognised the BCR-ABL kinase-inhibitor-insensitive leukemic stem cells in chronic myeloid leukemia.[10] The presence of the cancer stem cells is a determining factor for the growth and progression of the any tumor, its metastatic activity, and also sensitivity to therapy. In this regards, CML immature blast cells with the leukemic stem cell-like phenotype also must be resistent to imatinib-therapies. Therefore, other mechanisms in prevention of LSC imatinib-resistance is an urgent issue, the solution of which will help to deepen knowledge about the biology of tumor growth in part, CML blast crisis biology. Our new strategy of LSC imatinib-resistance causal binding with epithelial-to-mesenchymal transition (EMT) mechanism in CML blast crisis biology.[11] EMT generates cells with properties of stem cells and therefore contributes to cancer stem cell progression resulting in tumor resistance.[12-14] Although EMT has been studied in relation to epithelium-derived tumors, there is increasing evidence implicating the involvement of EMT activators in hematopoietic malignancies.[15] The expression of some EMT modulators has been demonstrated in CML Ph+

leukemia cells.[16]

The pivotal EMT activator is transcription factor Snail that has the most important role in maintaining stemness properties in tumor progression.[17,18] Therefore, overexpression of Snail can be a biomarker of poor clinical outcome for patients with cancer. In its turn, Snail silencing effectively suppresses tumour growth and invasiveness.[19] Nonetheless, the role of Snail in leukemia remains obscure[20,21] and deserves more attention as targeting EMT- modulator in CML imatinib-resistance therapy. In our recent study by real-time RT-PCR analysis have been affirmatively found the presence of overexpression of transcription factor Snail as the leukemic stemnes marker and EMT- inducer in CML blast crisis K562 cells and its efficient down-regulation by vitamin E.[22] In addition, earlier, we also first revealed that vitamin E, alpha-tocopherol, might be an effective inducer of major myeloid master regulator C/EBP alpha transcription factor and consequently requared of G-CSFR (granulocytic - colony stimulated factor receptor) for therminal granulocytic differentiation potential in CML blast crisis K562 cells in vitro.[23] The loss of master regulator C/EBP alpha is the major paradigm of leukemogenesis with the block of myeloid differentiation in CML and AML cells.[24-26] In its turn, restoration of C/EBPalpha expression in a BCR-ABL+ cell line induces terminal granulocytic differentiation.[27] Moreover, we determined the inverse relationship between vitamin E-dependent Snail - EMT suppression and restored myeloid master regulator C/EBPα in CML blast crisis K562 cells.[22] Therefore, in such observed vitamin E-modulating effects we found feedback between SNAIL-EMT phenotype and acquired C/EBPα - myeloid differentiation phenotype in K562 CML blast stem cell-like cells in vitro. In this connection we first observed the vitamin E capability to reverse of EMT stemnes phenotype to myeloid differentiation potential pathway in K562 blast crisis CML cells. In the present study we further deepenig understand of CML blast crisis biology as EMT-dependent mechanism of imatinib-resistance. The aim of the present study is to compare the effects of both vitamin E and imatinib on gene expression level of transcription factors SNAIL EMT-inducer and C/EBPα major myeloid master regulator in CML blast crisis K562 cells in vitro.

MATERIALS AND METHODS

K562 cell line originated from a CML patient in blast crisis was obtained from Depository of Cell Lines and Tumor Strains of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, the NAS of Ukraine. The cells were grown in suspension in RPMI-1640 medium supplemented with 10% of fetal calf serum.

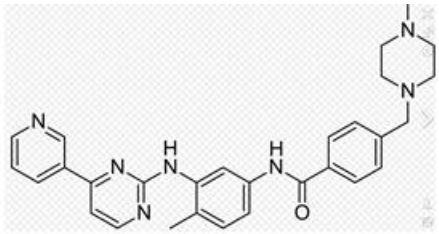
Valproic acid (VA; Pharma GMBX, Austria) and vitamin E (Technolog, Ukraine) were used for the study. RNA was isolated from cultured cells by means of TRIzol reagent (Invitrogen, Gaithersburg, MD) according to manufacturer's recommendations. RNA concentration in samples was measured on Nanodrop 2000 spectrophotometer (Termo Scientific, USA). RNA was converted to cDNA using the Qiagen's QuantiTect Rev. Transcription Kit (Qiagen, Hilden, Germany).

C/EBPα and G-CSFR mRNA expression was quantified by real-time RT-PCR using SYBR Green protocol. RT-PCR reactions were carried out using HotStarTaq DNA polymerase (Qiagen), 50 ng of cDNA and SYBR Green in a 1:60,000 dilution in triplicate. PCR conditions were: 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 on an Bio-Rad Real-time PCR Detection System IQ5, CA. Real-time RT-PCR was performed and conducted by means of SYBR Green (BioLab) on termocycler CFX96 Real-Time System (Bio-Rad, USA) for C/EBPα and G-CSFR gene mRNA expression level detection.

The primers of corresponding genes was used as: C/EBPα forward: 5´-CAAGAACAGCAACGAGTACCG-3´; reverse: 5´-GTCACTGGTCAACTCAGCAC-3´; G-CSFR forward — 5´-ACAAGCCGCAGCGTGGAGAAG-3´; reverse — 5´-TTCTGAAGGCAGGTGGAAGGTG-3´. SNAIL forward: 5′-CAGACCCACTCAGATGTCAA-3′ ; reverse: 5′-CATAGTTAGTCACACCTCGT-3′ ; GAPDH is a reviewer gene, forward — 5´-CGCTCTCTGCTCCTCCTGTT-3´; reverse — 5´-CCATGGTGTCTGAGCGATGT-3´. The gene expression was quantified using 2 Δ-Ct method with normalization to mRNA expression of GAPDH. Statistical significance of differences was evaluated by Student`s t-test.

RESULTS AND DISCUSSION

Imatinib mesylate (STI-571) is the standard of care in CML. Imatinib (also known as "Gleevec" or "Glivec"), a tyrosine kinase inhibitor, was called as "magical bullet," when it revolutionized the treatment of chronic myeloid leukemia (CML) in 2001.[28] The use of tyrosine kinase inhibitors as imatinib (Figure 1) became the breakthrough in therapy of the chronic myeloid leukemia (CML).[29]



Imatinib (Glivek): C29H31N7O

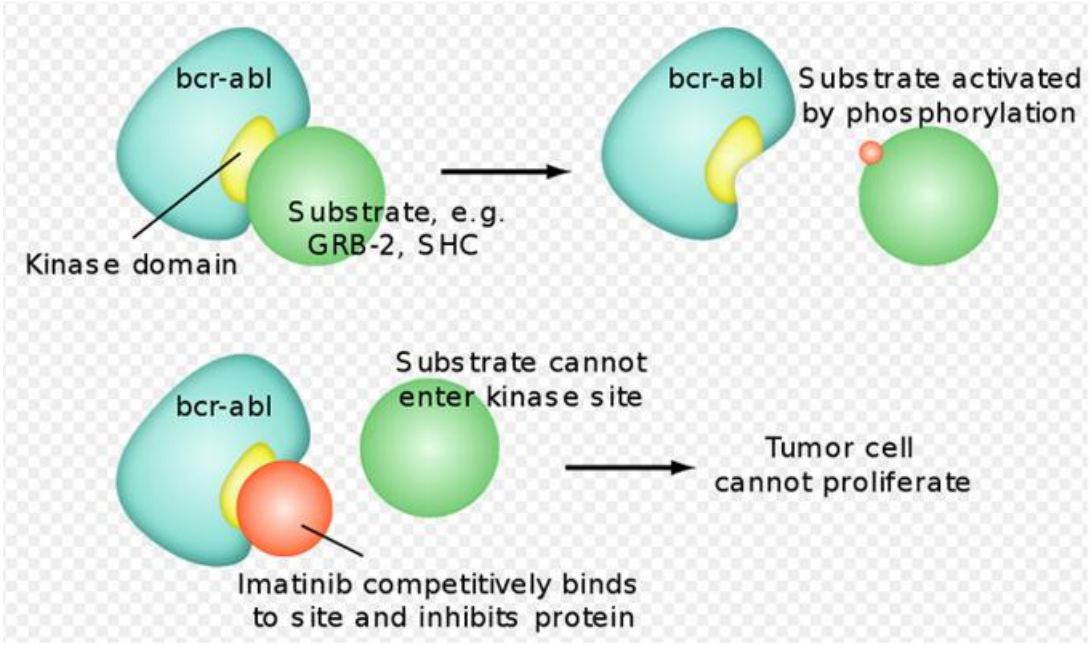


Figure 1: Mechanism of action of imatinib: competitively binds to Bcr-Abl kinase domain site and inhibits Bcr-Abl protein partners (Wikipedia).

Nevertheless however, the development of the resistance remains the major problem in clinical application of imatinib[30] and tyrosine kinase inhibitors of new generations.[7] Human chronic myeloid leukemia stem cells (LSCs) are insensitive to imatinib despite inhibition of BCR-ABL activity as the driving force of leukemogenesis in CML.[31] Therefore, it is logical to assume, that LSCs are BCR-ABL kinase independent and are themselves the source of TKI resistance, relapse, or disease progression, which is another major area of need in CML treatment. Understanding LSC resistance to imatinib (TKI) is a critical area of investigation in CML biology.

In the present study we propose the alternative mechanism imatinib resistance, indeed BCR-ABL kinase independent. We found that imatinib develops two mechanisms: along with BCR-ABL- TKI activity imatinib observed the function in LSCs induction as source of imatinib resistance through imatinib-EMT activation mechanism. Vitamin E earlier we study was targeting EMT factor down-regulating gene expression of transcription factor SNAIL a major EMT- modulator and consequently up-regulating myeloid differentiation master regulator EBPα and G-CSFR.[22,23] Vitamin E study showed that both processes are related. The vitamin E-dependent repressive EMT mechanism induced myeloid differentiation mechanism through vitamin E-dependent reactivation C/EBP α master regulator in K562 CML blast cells. Therefore, in the present research imatinib was compared with vitamin E on the affecting gene expression SNAIL EMT-inducer and C/EBP α myeloid transcription factor in CML blast crisis K562 cells in vitro by real-time RT-PCR assay.

On the data obtained we found vitamin E as a new factor directed toward removing the myeloid differentiation block in CML blast cells. Indeed, we have not found detectable expression of C/EBP α as major myeloid master regulator in CML blast crisis K562 cells. In this connection, we have shown vitamin E-dependent reactivation of myeloid differentiation master regulator C/EBP α and consequently granulocytic-colony stimulated factor receptor (G-CSFR) in K562 blast cells in vitro. Upon 48-h culture with vitamin E at a dose of 100 μM, K562 cells expressed both myeloid gene C/EBP α and G-CSFR (Figure 2).

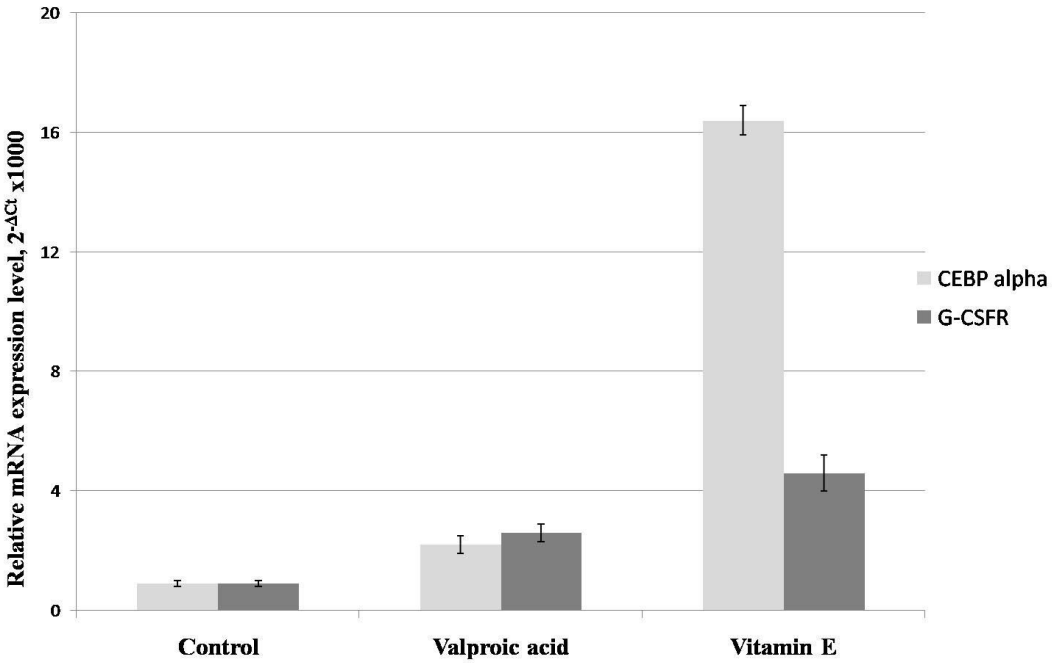


Figure 2: The relative levels of C/EBPα and G-CSFR mRNA expression in K562 cells exposed to valproic acid (2 mM) or vitamin E (100 µM) for 48 h.

A therapeutic approach directed toward removing the differentiation block was in fact perceived as a more specific therapy than the traditional treatments based on cytotoxic drugs. Several chemicals such as dimethyl sulfoxide, sodium butyrate, valproic acid, etc. have been demonstrated to induce not only apoptotic death but also the differentiation of CML cells in vitro.[32] Nevertheless, these substances were not specific enough targeting the cell impairments underlying CML and were used mostly for elucidating the mechanisms of CML cell differentiation induced in the experimental setting.

The question was raised whether imatinib as motivate BCR-ABL tirosine kinase inhibitor, could unblock differentiation in CML blast cells. Indeed, in our study took place the positive effect of imatinib on C/EBP α gene expression upon 48-h culture with imatinib at a dose of 2,5 mM in CML blast crisis K562 cells (Figure 3).

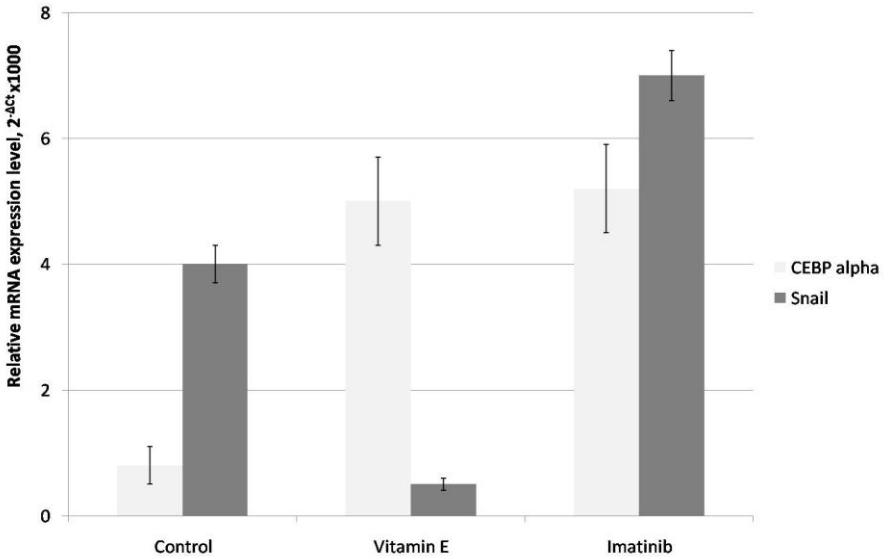


Figure 3: The relative levels of C/EBPα and Snail mRNA expression in K562 cells exposed to vitamin E (100 µM) or imatinib (2.5 mM) for 48 h.

However, the development of imatinib-resistance remains the major problem in clinical application of imatinib. Also the model CML blast crisis K562 cells are imatinib-resistance.[33] Therefore, it is contradicted any possibility to remove bloking of differentiation in CML cells by imatinib treatment. In this connection we propose that observed fact of imatinib-dependent visible increasing of C/EBP α gene expression is the simple reduction in the ration of BCR-ABL/BCR cells in CML progression. Moreover, in support of this opinion we confirmed the imatinib-resistance mechanism through up-regulation of SNAIL- EMT -inducer gene expression under imatinib exposure (2,5 mM) in K562 CML blast crisis cells (Figure 3). Therefore, up-regulation of SNAIL EMT-inducer by imatinib is not compatible with C/EBPα - unblock myeloid differentiation mechanism in CML cells K562. In contrast to imatinib, we have suggested the vitamin E-dependent down-regulation of SNAIL-EMT-inducer gene expression in K562 CML blast crisis cells in vitro (Figure 3). Consequently, SNAIL - EMT phenotype in CML cells determines the non possibility further C/EBPα activation mechanism in unblock myeloid differentiation potential for CML blast crisis K562 cells. Indeed, in contrast to imatinib vitamin E-dependent reactivation of C/EBPα gene expression causal binding only with vitamin E-dependent SNAIL-EMT repression in CML blast K562 cells (Figure 3). On the data obtained exclusively we have compared oppositive mechanisms of vitamin E and imatinib with regard to targeting EMT phenotype in CML blast cells. Vitamin E targeted EMT phenotype in K562 CML blast cells through its inhibitory action on SNAIL gene expression. In contrast, we shown that imatinib maintaining EMT phenotype in K562 blast cells through its activating action on SNAIL gene expression confirming imatinib – EMT resistance mechanism. Therefore, our results quite consistent with important reference by *Puissant A., Dufies M., Fenouille N., Sahra I.N., et al. (2012)* wherein "Imatinib triggers mesenchymal-like conversion of CML cells associated with increased aggressiveness".[34]

CONCLUSION

On the data obtained, we found the imatinib – EMT-resistant mechanism in CML blast crisis progression through affirmatively stimmulation of SNAIL-EMT phenotype.

Vitamin E we found versus imatinib is the potential repressor of SNAIL-EMT phenotype in CML blast crisis progression and consequently myeloid master regulator C/EBP α re-activation was taken place. Only in such feedback context between EMT phenotype and myeloid differentiation potential in CML blast cells our results establish a relationship between EMT phenotype and CML cells block myeloid differentiation. On this basic understanding our results in its turn is consistent with other authors by *Lourenço A.R., Roukens M.G., Seinstra D., et al. (2020)* suggesting that C/EBPα is crucial determinant of epithelial maintenance by preventing epithelial-to-mesenchymal transition.[35] Therefore, we conclude that vitamin E might be reversal remodelling factor from EMT phenotype to myeloid differentiation potential pathway in prevention of CML blast crisis imatinib-resistance. In conclusion, our present research achievement in imatinib-resistance mechanism is related to imatinib-dependent EMT- resistance during LSCs induction. In such connection, LSCs are not imatinib independent source imatinib-resistance.

We have proposed that vitamin E (alpha-tocopherol) can be used appearently anti-EMT stemnes factor in CML blast crisis progression with LSC phenotype poor resolving in CML imatinib-resistant therapy.

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