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THE EFFECT OF VITAMIN E AS DIFFERENTIATION-LIKE FACTOR IN CML BLAST CRISIS K562 CELLS.

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ABSTRACT

Background: Chronic myelogenous leukemia (CML) is a clonal hematopoietic stem cell disorder associated with the activity of *BCR-ABL* fusion oncogene that arises from the reciprocal translocation t(9;22)(q34;q11). The molecular events underlying the transition from the chronic phase to the blast crisis are still poorly understood. The transcription factor C/EBP-alpha (CCAAT/enhancer-binding protein α) implicated as an inhibitor of cell proliferation and a master regulator of hematopoietc stem cell (HSC) myelopoiesis plays a critical role in granulocytic differentiation. The progression of CML to blast crisis is correlated with down-modulation of myeloid C/EBP-alpha transcription factor. Therefore, C/EBP-alpha may be considered as a putative target in differentiation therapies in myeloid leukemias as CML and AML. **The aim** of the study was to assess the potential of vitamin E as the possible inducer of C/EBP-alpha expression in BCR-ABL-positive chronic myelogenous blast crisis (BC-CML) K562 leukemic cells. **Methods:** RNA extracted from CML blast crisis K562 leukemic cells was converted to cDNA and real-time RT-PCR was performed using SYBR Green protocol with primers to C/EBP alpha and G-CSFR genes. Samples were cycled using the standard SYBR Green protocol on Q5 Bio-Rad StepOne qPCR instrument and analyzed using the comparative cycle threshold (*C*T) method to obtain relative mRNA expression. To analyze the modulation of expression of C/EBP alpha and G-CSFR, CML cells were incubated with 100 µM vitamin E. **Results:** We have not found detectable expression of C/EBP-alpha in CML blast crisis K562 cells. Upon 48-h culture with vitamin E at a dose of 100 µM, K562 cells expressed both C/EBP-alpha and G-CSFR (granulocyte colony-stimulate factor receptor) gene expression. **Conclusion:** Vitamin E restored the expression of C/EBP-alpha mRNA in CML blast crisis K562 cells. In this setting, G-CSFR expression in vitamin E treated K562 cells seems to point out to the activation to granulocytic differentiation. It should be further elucidated whether such effects of vitamin E on C/EBP-alpha transcription factor are direct or mediated through generation of indirectly due to antioxidant properties of vitamin E.

KEYWORDS: C/EBP alpha and G-CSFR, CML cells.

INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal malignacy of a hematopoietic stem cells (HSCs) caused by a reciproca translocation t(9:22) (q34;q11), fusing the Bcr gene to Abl gene[1,2] The p210 BCR-ABL fusion protein has elevated ABL tyrosine kinase activity that is crucial for transformation of HSCs.[3] The disease begins with an indolent chronic phase (CP) that can last for 3 to 5 years. If untreated, it progresses into accelerated phase (AP) and within a year, blast phase (BP). Survival at this point is less than 1 year during disease progression, mutations and the Philadelphia chromosome (Ph) appear as a process called clonal evolution. It is the development of the blast crisis that determines the poor prognosis of the disease BC-CML. Bcr-Abl tyrosine kinase ingibitots (TKIs), imatinib, nilotinib and dasatinib, are the current treatmet of chronic myeloid leukemia, but BCR-ABL driving point mutations are the principal casse of resistance to the treatment[4,5] Thus, CML leukemic stem cells (LSCs) as currieers of oncoprotein p210-BCR-ABL tyrosine kinase after its effective inhibition still save the ability to be source for resistant/relapce disease. Imbalance between kinase and phosphotase activities is critical in many hematological malignancies[6] Oncogenic kinases and tumor suppressor phosphatases maintain the cell homeostasis by exerting their activities on cell growth, survivat and differentiation. It was shown that in BCR-ABL transformed LSCs and CML blast crisis progenitors, the phosphatase activity of the tumor suppressor PP2A is crucial inhibited[7,8] (Fig 1).

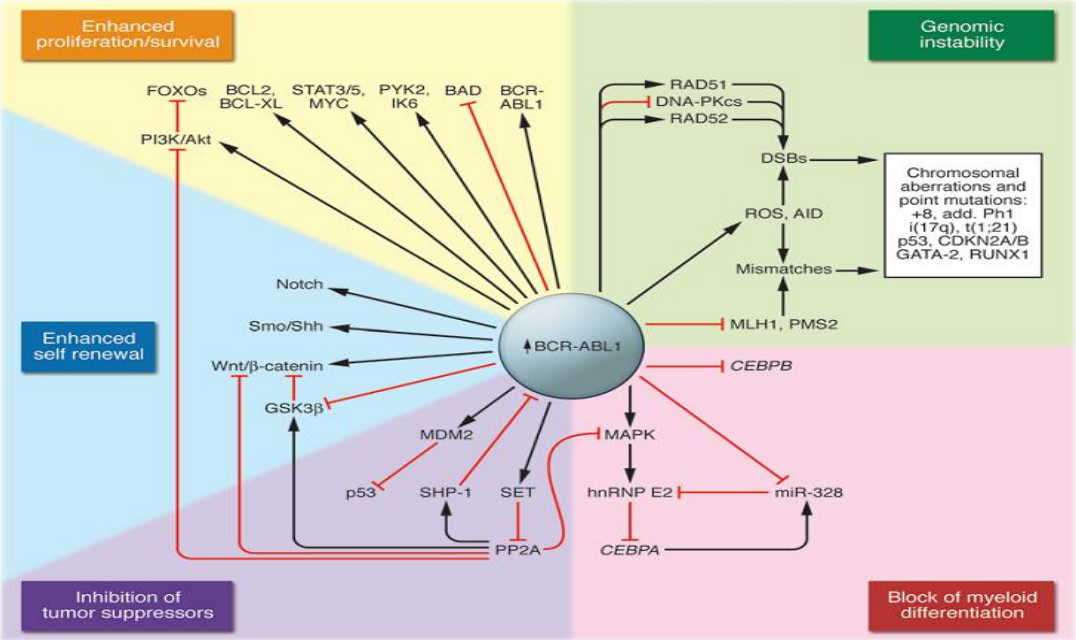

Figure: 1. BCR-ABL oncoprotein in Intracellular mechanisms.[3]

The pharmacological activation of PP2A suppresses BCL/ABL activity and induces BCR/ABL degradation. Furthemore, PP2A leads to growth suppression, enhanced apoptosis, restored differentation of cancer cell. Along this there are different classes of PP2A activating compounds and Vitamin E (α - tocopherol) among them.[9] In this regard, in this research, we evaluated the effect of Vitamin E action as well as well known activator of PP2A, on the crucial factors of myeloid differentiation, C/EBP α and G-CSFR, expression of which in CML (chronic phase) and during CML blast crisis is drastically decreased.[10,11]

MATERIALS AND METHODS

In the study is used the CML blast crisis K562 leukemic cell line exposed to vitamine E in the concentration 100 μM and valproic acid in the concentration 4 mM upon 48 h culture. RNA was isolated from cultured cells by means of Trizol-reagen (Sigma - Aldrich)) in obedience to protocol. The concentration of standards of RNA was measured on Nanodrop 2000 spectrophotometer (Termo Scientific, USA). The synthesis of cDNA was conducted during a concentration to RNA of from 300 to 400 ng/μл on standard methodology with the use of reverse transcriptase enzyme. Real-time RT-PCR was performed and conducted by means of SYBR Green (BioLab) on termocycler CFX96 Real-Time System (Bio - Rad) for C/EBP alpha and G-CSFR gene mRNA expression level detection. The primers of corresponding genes was used as: C/EBP alpha

forward: 5`- CAA GAA CAG CAA CGA GTA CCG-3`; reverse: 5`- GTC ACT GGT CAA CTC AG CAC-3`; G-CSFR forward - 5′- ACAAGCCGCAGCGTGGAGAAG - 3′;reverse - 5′- TTCTGAAGGCAGGTGGAAGGTG - 3′. GAPDH as a refferent gene: forward - 5 '- CGCTCTCTGCTCCTCCTGTT - 3'; reverse - 5 '- of CCATGGTGTCTGAGCGATGT — 3';

The quantitative results was conducted by 2 Δ-Ct method analyzed in triplicates.

Statistical significance of differences was evaluated by Student`s *t*-test.

RESULTS AND DISCUSSION

Hematopoietic stem cells (HSCs) differentiation is regulated by lineage-specific transcription factors. It has recognized that loss of transcription factor CCAAT-enhancer binding protein α (C/EBPα) expression or function may contribute to the CML differentiation block, enhanced proliferation, and development of acute myelogenous leukemias (AMLs).[12] C/EBPα is mainly involved in cell fate decisions for myeloid differentiation.[13] Therefore, transcription factor CCAAT/enhancer binding protein α (C/EBPα) is a crucial master regulator of myeloid differentiation in part directing granulocyte and monocyte differentiation [Fig. 2,[14]].

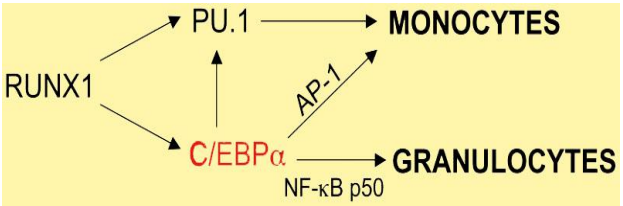

Figure: 2. Schematic model for the transcriptional control of myeloid development by transcription factors RUNX1, C/EBPα, and PU.1.[14]

C/EBPα directly activates transcription of granulocyte colony-stimulating factor receptor (G-CSFR) during common myeloid progenitor (CMP) lineage committing

activation.[15-17] Therefore, C/EBPα loss is connected with early myeloid maturation block in CML. Thus, the malignant transformation in CML and AML is associated with leukemic stem cells (LSCs) progression which reflect the loss of C/EBPα that causal links with repression of G-CSF receptor (G-CSFR) as direct neutrophyl/ granulocytic terminal differentiation marker. Therefore, C/EBP alpha is major transcription factor as myeloid master regulator.[18,19] The finding of C/EBP*α* alterations in subgroups of acute myeloid leukemia patients suggests a direct link between critically decreased C/EBP*α* function and the development of the disorder.[20] Tavor et al[21] first shown that the restoration of C/EBP-alpha expression in BCR-ABL-positive KCL22 leukemic blast cells transfected with *C/EBPα plasmide vector(pMTα)* triggered differentiation along with a proliferative arrest, a block in the G2/M phase of the cell cycle and a gradual increased in apoptosis. Therefore, C/EBP-alpha may be considered as a putative target in differentiation therapies in myeloid leukemias. The aim of the present study was to assess the potential of vitamin E as the possible inducer of C/EBP-alpha expression in BCR-ABL-positive chronic blast crisis myelogenous leukemia K562 cells. At the figure 2 is shown the mRNA level C/EBP alpha gene expression in CML blast crisis K562 leukemic cells under vitamin E exposue (100 µM) compared to control K562 and valproic acid exposure (4mM) that recently reffered in leukemia therapies.[22]

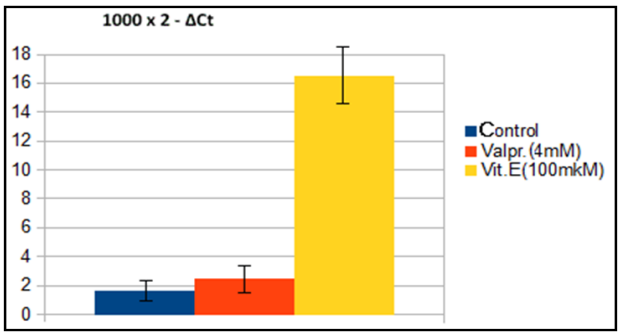

Figure: 2. The relative levels of mRNA C/EBP-alpha gene expression under Valproic acid (4mM) and Vitamin E (100 µM) exposure of K562 CML blast stem cell line along of 48 h.

We have not found detectable expression of C/EBP-alpha in K562 cells upon 48-h culture (Fig. 2, Control). On the data obtained on the three independent experiments (n=3) we revealed the Vitamin E-dependent induction of C/EBP-alpha transcription factor gene expression on the mRNA relative level as 16,516 ($p<0,05$) compared to Control K562 as 1,6770 ($p<0,05$) and Valproic acid as 2,524 ($p<0,05$) (Fig. 2). K562 cells exposured by 100 µM of vitamin E and by 4 mM of valproic acid upon 48-h culturation. Also, we revealed that valproic acid action was not significantly in contrast to vitamin E action. Probably, valproic acid have another mechanisms as histone deacetylase inhibitor (HDACi) in leukemogenesis in part likely epigenetic then signaling.[16] On these results we emphasized that vitamine E triggeres K562 CML blast cell differentiation potential through vitamin E-dependent activation of C/EBP-alpha as myeloid master regulator. Moreover, we have detected the C/EBP alpha - induced increasing of terminal granulocytic/neutrophilic differentiation marker G-CSFR gene mRNA expression relative level as 4,613 ($p<0,05$) in CML blast crisis K562 cells under vitamin E exposure along of 48 h compared to control K562 as 0,898 ($p<0,05$) and valproic acid as 2,541 ($p<0,05$) accordingly (Fig. 3).

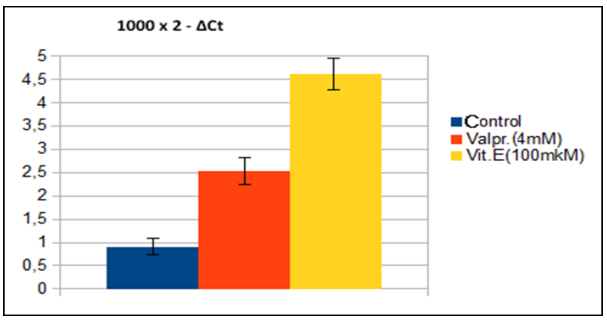

Figure 3. The relative levels of mRNA G-CSFR gene expression under Valproic acid (4mM) and Vitamin E (100 mkM) exposure of K562 CML blast stem cell line culture along of 48 h;

As see on Figure 3, on the three independent experiments (n=3) these results also suggested a greater increasing mRNA G-CSFR gene expression by vitamin E compared with Control than effect of valproic acid action on G-CSFR gene expression compared with Control. Moreover, we propose that vitamin E effect on increasing of G-CSFR gene expression is mediated through vitamin E-dependent C/EBP-alha induction.

In the Table is presented the statistic values of C/EBP alpha and G-CSFR gene mRNA expression relative levels (n=3) in CML blast crisis K562 leukemic cells under vitamin E exposure (100 µM) upon 48-h cultured.

Table. Fold increase (analyzed in triplicates) in gene expression in K562 cells line culture under 48 h vitamin E exposure (100 µM) calculated by $2^{-(\Delta\Delta Ct)}$ method.

N (n=3)	C/EBP alpha		G-CSFR	
	Fold increase	Standard Deviation, σ	Fold increase	Standard Deviation, σ
1	8.395 ± 1.481	1.219	3.930 ± 1.843	1.988
2	9.854 ± 0.023	1.219	4.626 ± 0.853	1.988
3	11.381 ± 1.506	1.219	5.134 ± 1.357	1.988

$\sigma = \sqrt{1/n \sum(x_i - \bar{x})^2}$

On the data obtained we have concluded that Vitamin E can restore the expression of C/EBP-alpha myeloid master regulator in CML blast crisis K562 leukemic cells. In this setting, we have determined the C/EBP alpha-dependent activation of G-CSFR gene expression through vitamin E -dependent induction of C/EBP alpha transcription factor in vitamin E treated CML blast crisis K562 leukemic cells. Also, valproic acid was appeared less effecting stimulator, what vitamin E action as we shown. It should be further elucidated the vitamin E mechanism whether such effects of vitamin E on myelogenic transcription factor C/EBP-alpha are direct or mediated through generation of indirectly due to antioxidant properties of vitamin E. Nevertheless, on the data obtained we first offer vitamin E-associated certainment myeloid differentiation-like potential for clinical utility (Fig. 4).

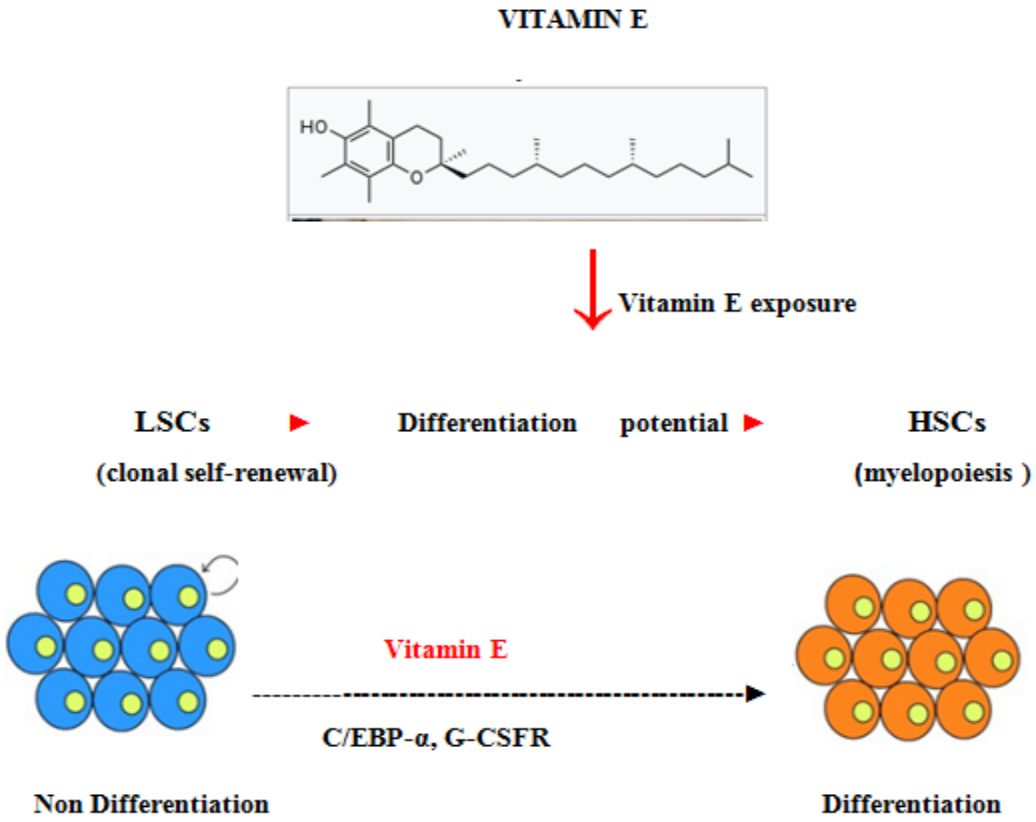

Figure: 4. The Schematic model in Challenge for Vitamin E in CML Leukemia Treatment. Vitamin E activates C/EBP-α and G-CSFR for myeloid progenitor cells differentiation.

This model may provide our proposal that additional vitamin E role is required for targeted loss leukemic stem cell phenotype because vitamin E may to play a critical role in regulating the balance between leukemic stem cells (LSCs) and hematopoietic stem cells (HSCs) through differentiation potential stimulation by vitamin E-dependent myeloid master regulator C/EBP alpha and consequently G-CSFR as we studied in CML blast crisis K562 leukemic cell line.

CONCLUSION

Because CEBP-alpha exhibit tumor suppressor properties in human leukemogenesis, differentiation therapy of CML and AML leukemias is based on restoring CEBP-alpha function as it certain role in myelopoiesis in general and granulopoiesis in particular. We shown a new peculiar insight of vitamin E as differentiation-like factor through restoring CEBP-alpha function in CML blast crisis K562 cells. On the data obtained we conclude that Vitamine E–dependent induction of C/EBP alpha and G-CSFR as pivotal myeloid differentiation markers can be proposal for differentiation therapies in warning CML blast crisis progression.

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