ejpmr, 2020,7(12), 162-169

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EMT TARGETING IN CML BLAST CRISIS K562 CELLS BY VITAMIN E: BLOCKING EMT-SNAIL TRANSCRIPTION FACTOR AND UNBLOCKING MYELOID MASTER REGULATOR CEBP α TRANSCRIPTION FACTOR

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Article Received on 13/10/2020 **Article Revised on 03/11/2020** **Article Accepted on 23/11/2020**

BACKGROUND

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). The persistence of leukemic stem cells (LSCs) remains a major obstacle to cure CML. Epithelial mesenchymal transition (EMT) mechanism is known to contribute to LSC tumor progression. Although EMT has been studied in relation to epithelium-derived tumors, there is increasing evidence implicating the involvement of EMT activators in hematopoietic malignancies. The expression of some EMT modulators has been demonstrated in Ph+ leukemia cells. EMT-inducer Snai1 has the most important role is in maintaining stemness properties in tumor progression. Nevertheless, the role of Snai1 in hematopoiesis and leukemia remains obscure. Earlier, we revealed that alpha-tocopherol might be an effective inducer of mRNA CEBP alpha in CML blast crisis K562 cells in vitro (Shvachko L.P., et al., 2018). **The aim:** of present study is to determine the relationship between Snai1-EMT suppression and restored CEBP alpha myeloid differentiation potential in CML cells induced by vitamin E (alpha-tocopherol). **Materials and Methods:** K562 cells were exposed to alpha-tocopherol (100 µM) or metformin (4 mM) for 48 h. RNA was extracted and converted to cDNA; RT-PCR reactions were carried out according to SYBR Green protocol detection using HotStarTaq DNA polymerase with primers for Snai1 and C/EBPalpha genes. The gene expression was quantified using 2-ΔCt method with normalization to mRNA expression of GAPDH gene. **Results:** We have found highly detectable Snai1 mRNA expression and down-regulated CEBP alpha in K562 cells. Vitamin E, alpha-tocopherol, targeted suppressed Snail mRNA expression. Such suppression of Snail inversely is correlated with restored CEBP alpha mRNA expression by vitamin E and enhanced by metformin pointing to the possible synergistic effect with vitamin E effect. **Conclusion:** We observed that several modulators of gene expression affect Snail1 and CEBP alpha mRNA expression in K562 cells in different directions. In particular, vitamin E (alpha-tocopherol) down-regulates Snail1 and up-regulates CEBP alpha. One could suggest the causal relationship between Snai1 suppression and restoration of CEBP alpha expression that seems to contribute to recover myeloid differentiation potential of CML blast cells. From one side, this study have suggested that Snai1 EMT-inducer contribute to pathogenesis of CML blast crisis. On the other hand we revealed that vitamin E can restore normal HSC myelopoietic function during SNAIL repression. Therefore, we have proposed that vitamin E - alpha-tocopherol can be used in anti-EMT stemnes therapy for clinical CML blast crisis progression with LSC phenotype which recently poor resolving.

INTRODUCTION

The epithelial–mesenchymal transition (EMT) is the process of conversion of cells from a differentiated epithelial state into a dedifferentiated migratory mesenchymal stem cell phenotype, which is crucial for regulatory mechanisms in embryogenesis, organ fibrosis and cancer metastasis.[1,2] EMT induction in cancer cells results in the acquisition of invasive and metastatic properties through EMT-induced formation of CSCs.[3,4] Therefore, EMT programme is a critical regulator of the CSC phenotype. In particular, EMT contributes to therapy resistance through EMT-induced formation of CSCs.[5,6] The potential for novel EMT therapeutics overcoming drug resistance is increased.[7-9] One of the major triggering events for EMT is the activation of EMT-transcription factors (TFs), such as Snail, Twist, zinc finger E–box binding homeobox (ZEB) famalies.[10,11] Transcription factor Snail is a prominent inducer of EMT[12,13] because Snail is a strong direct repressor of epithelial E-cadherin expression marker in tumor cells.[14] Snail belongs to the Snail superfamily of zing-finger transcription factors considered essential for the initial induction of EMT in tumor metastasis.[15] In turn, Snail silencing effectively suppresses tumour growth and invasiveness.[16] Therefore this places transcription factor Snail in a central position in EMT process in cancer progression. EMT has received increasing attention in hematological malignancies.[17] As

in solid tumors, EMT-TFs are also implicated in cancer proliferation, stemness, anti-apoptosis and drug resistance in hematological malignancies. The evidence shows that EMT-TFs are related to chemotherapy and radiotherapy resistance in both myeloid and lymphoid malignancies.[18] These findings make EMT-TFs potential targets for combination with conventional treatments. For example, the Snail may control self-renewal, anti-apoptosis and treatment resistance in leukemia, which makes Snail transcription factor a potential target for leukemia treatment.[19,20] Accumulated evidences by Carmichael C.L., et al, (2016), confirm the importance of Snail EMT-inducer in AML development and pathogenesis.[20] In this report transgenic Snail1 expression induces myeloid leukaemia in mice,[3] and human AML cells show increased Snail 1 expression compared to haematopoietic stem and progenitor cells (HSPCs).[20] Taken together, regulation of the epithelial to mesenchymal transition (EMT) is an emerging theme in chronic myeloid leukemia (CML) biology and novel strategies in its therapies. Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell (HSC) disorder associated with the activity of BCR-ABL fusion oncogene due to the reciprocal translocation t(9;22)(q34;q11) that is characterized by an increased growth of abnormal undifferentiated myeloid progenitor cells within the bone marrow.[21, 22] Leukemia stem cells (LSCs) are the root of chronic myeloid leukemia.[23,24] Recently, imatinib, an inhibitor of BCR-ABL, has emerged as the leading compound to treat CML patients but despite encouraging clinical results, resistance to imatinib represents a major drawback for therapy.[25] It is reffered that imatinib triggers epithelial-mesenchymal transition (EMT)-like phenotype as Mesenchymal-Like Conversion of CML Cells with Leukemic stem cells (LSCs) phenotype resulting in imatinib resistance and folowing increased aggressiveness.[26] CML LSCs do not depend on BCR-ABL signaling for their survival [27], and their persistence remains a major obstacle to curing CML. Indeed, leukemia stem cells (LSCs) are considered responsible for leukemia initiation, relapse and resistance to chemotherapy.[28, 29] If LSCS are the root of chronic myeloid leukemia (CML)[24] then EMT inducing LSCs formation is reason root of CML. Therefore, EMT-induced LSC formation is an emerging axis of evil in the war on leukemia. Earlier we also suggested the overexpression EMT-inducers Snail1, Twist1 and N-cadherin in patients with CML and AML myeloid malignancies which associated with leukemic stem cell (LSC) phenotype.[30] Moreover, earlier we observed the differentiation-like potential of vitamin E in exposured CML blast crisis K562 cells through vitamin E-dependent reactivation of CEBP alpha master regulator of myelopoiesis/granulopoiesis.[31] Since the pivotal hallmark of BCR-ABL – positive leukemias as CML is blockage of myeloid differentiation[19] we have aimed in this study to investigate the relationship between targeting Snail transcription factor as muster EMT-inducer and restoring CEBP alpha transcription factor as muster regulator of myeloid differentiation in blast crisis CML where undifferentiated myeloid progenitors are candidate leukemic stem cells (LSCs) in uncontrolled accumulation of stem-like blasts losing CEBP alpha.[32] C/EBPα is a critical regulator of myeloid cell development directing granulocyte and monocyte differentiation.[33,34] The level of C/EBPα expression was significantly declined in CML patients.[35] The loss of C/EBPα transcription factor expression or function may contribute to the differentiation block, enhanced proliferation, and development of acute myeloid leukemia (AML).[36,37] Therefore, C/EBPα deregulation is declared as a paradigm for leukemogenesis.[37] In turn, restoration of C/EBP alpha expression in a BCR- BL$^+$ Cell line induces terminal granulocytic differentiation.[38] Therefore, C/EBPα may be considered as a putative target in differentiation therapies in myeloid leukemias.[31,38] Our present study focused on the causal binding between EMT inhibition and consequently restored myeloid differentiation potential in CML blast crisis leukemic K562 cells. To achieve this goal we have aimed to study the modulation potential of vitamin E in the relationship between Snail EMT–inducer and CEBP alpha myeloid muster regulator in CML blast crisis K562 leukemic cells in vitro investigation. We use vitamin E as modulator in this study based on the our previous results on vitamin E role[31] and also taking into account the important report by Nieborowska-Skorska et al. (2013) demonstrating that vitamin E prevents accumulation of imatinib-resistant BCR-ABL1 kinase mutations in CML.[39] Metformin as emerging anti-cancer drug in particular with anti-EMT effects.[40,41] we used to compare with the effects of vitamin E in our study.

MATERIALS AND METHODS

CML blast crisis K562 leukemic cell line was used. K562 cell line originated from a CML patient in blast crisis phase was obtained from Depository of Cell Lines and Tumor Strains of the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, the National Academy of Sciences of Ukraine. The cells were grown in suspension in RPMI-1640 medium supplemented with 10% of fetal calf serum. Vitamin E (alpha-tocopherol,Technolog, Ltd., Ukraine) was added to culture medium in a final concentration of 100 μM. Total RNA was extracted using TRIzol (Invitrogen, USA) according to the manufacturer's instructions. RNA was converted to cDNA using QuantiTect Reverse Transcription Kit (Qiagen, Germany). Real-time reverse transcription polymerase chain reaction (RT-PCR) was performed using SYBR Green protocol. RT-PCR reactions were carried out using HotStarTaq DNA polymerase (Qiagen, Germany), 50 ng of cDNA and SYBR Green in a 1:60,000 dilution in triplicate. PCR conditions were as follows: 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 iQ5 Real-time PCR Detection System (Bio-Rad, USA). We performed the real time RT-PCR for mRNA expression of transcription

factors Snail and CEBP alpha in the blast crisis CML leukemic cell line K562 without and under exposure by vitamin E (100mkM) and metformin (4mM) along 48h. RNA extracted from K562 cells whithout and cultured with vitamin E or metformin was converted to cDNA, RT-PCR reactions were carried out with SYBR Green protocol detection using HotStarTaq DNA polymerase with primers for Snail gene as forvord: 5′-CAGACCCACTCAGATGTCAA-3′; reverse: 5′-CATAGTTAGTCACACCTCGT-3′; and C/EBP alpha gene as forvord:5´-CAAGAACAGCAACGAGTACCG-3´; reverse: 5´-GTCACTGGTCAACTCCAGCAC-3´; GAPDH gene as refferent: forward — 5´-CGCTCTCTGCTCCTCCTGTT-3´; reverse — 5´-CCATGGTGTCTGAGCGATGT-3´. The gene expression was quantified using $2^{-\Delta Ct}$ method [42] with normalization to mRNA expression of GAPDH. Statistical significance of differences for 3 independent studies (n = 3 was evaluated by Student's *t*-test.

RESULTS AND DISCUSSION

Transcription factor CEBP alpha muster regulator of myelopoiesis/granulopoiesis is repressed in Bcr-Abl+ CML progression.[34-37] However the mechanisms underlying this down-regulation of CEBP alpha myelopoiesis muster regulator remain in general unknown. In the present study we point the reverse relationship mechanism between EMT phenotype and myeloid muster regulator C/EBPα in CML blast crisis progression in K562 cells. We have revealed the tightly reverse relationship between transcription factor Snail as initial EMT-inducer and transcription factor CEBP alpha as muster regulator of myelopoiesis in CML blast crisis leukemic K562 cells was investigated. In that suggestion we have found highly detectable Snail1 mRNA overexpression and down-regulated CEBP alpha mRNA expression in K562 cells by real time RT-PCR (Fig. 1, Fig. 2). Earlier our data suggest that vitamin E is able to restore the expression of C/EBPα mRNA in CML blast crisis K562 cells.[31] It should be further elucidated whether such effects of vitamin E on myeloid transcription factor C/EBPα are direct or mediated indirectly due to the anti-EMT effects of vitamin E in CML blast crisis K562. In the present study we observed the modulation effects of vitamin E (alpha-tocopherol) on repression of EMT-inducer Snail transcription factor that consequently restored myeloid transcription factor CEBP alpha in CML blast crisis K562 cells exposured by vitamin E (100 µM) for 48 h (Fig. 1, Fig. 2). Metformin exposured in K562 (40 mM) for 48 h was less effective than vitamin E in targeted Snail mRNA inhibition but together with vitamin E observed slightly increased effect vitamin E on CEBP alpha mRNA expression pointing to the possible synergistic effect (Fig. 2, Table 2). Metformin known emerging anti-cancer drug in particular with anti-EMT effect,[40,41] was used to comparisson with vitamin E effects in our study. On the data obtained we revealed that vitamin E was indeed more effective modulator of down-regulation of EMT-inducer Snail and up-regulation of myeloid muster regulator CEBP alpha then metformin. Taken together, we first shown that vitamin E, alpha-tocopherol, suppressed EMT-Snail mRNA expression and such suppression of Snail inversely is correlated with restored CEBP alpha mRNA expression in CML blast crisis K562 leukemic cells. The results from qRT-PCR showing the relative levels of mRNA Snail EMT-inducer expression in CML blast crisis K562 cells and in K562 cells exposured in vitro by vitamin E upon 100 µM for 48 h are presented at the Fig. 1, Table 1. Also, the results from qRT-PCR showing the relative levels of mRNA CEBP alpha myeloid transcription factor expression in CML blast crisis K562 leukemic cells and in K562 cells exposured in vitro by vitamin E upon 100 µM for 48 h as are presented at Fig. 2 and Table 2. Data represented as mean ± SD of three independent experiments (n=3). To quantify the obtained data, the comparative Ct method was used.[42] P-values were calculated by Student *t*-test as P<0,001 for vitamin E (100 mkM) effects and P<0,05 for metformin (4 mM) effects exposure in CML blast crisis K562 leukemic cells in vitro , relatively (Table 3, Table 4).

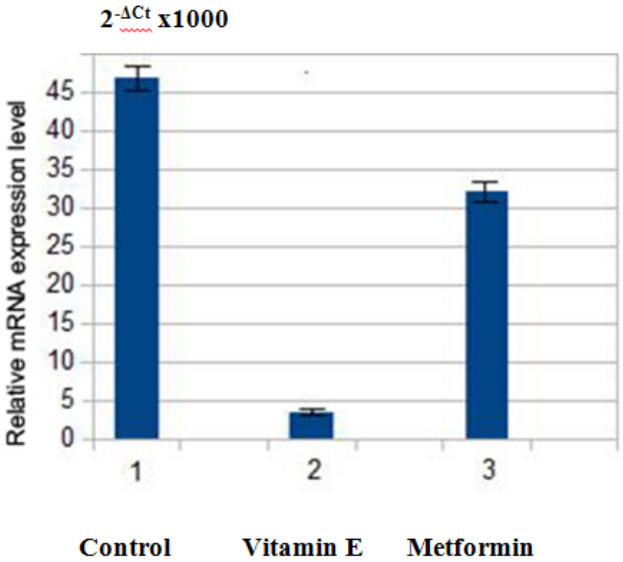

Figure 1. Relative mRNA expression level of transcription factor SNAIL as initial EMT-inducer in CML blast crisis K562 cells and its exposure by vitamin E (100 µM) and metformin (4mM) for 48 houers, calculated by 2- (ΔCt) method. 1 - Control K562; 2 - K562 + Vitamin E; 3- K562 + Metformin;

As see at Fig. 1, the overexpression of transcription factor SNAIL occurs in CML blast crisis K562 cells, suggesting EMT phenotype (1). The treatment CML blast crisis K562 cells by vitamin E (100 µM) drastic reduces Snail EMT-inducer expression on the mRNA level as was study by real-time RT-PCR (2). Metformin was less efficient than vitamin E during Snail EMT-inducer repression (3). In percentage, the relative level of mRNA expression of the EMT-inducer Snail gene in control K562 cells (1) is 100% whereas under vitamin E exposure (2) is 15,32% (p<0.001) that is corresponded to the percentage reduction on 84,68%, while under

metformin exposure (3) is 68,08% ($p<0.05$) that is corresponded to the percentage reduction only on 31,92%, respectively. Taken together, we have revealed that vitamin E is significantly more effective Snail-EMT repressor than metformin in the progression of CML blast crisis leukemic cell line K562.

Table 1. The Fold-decreasing of relative levels of the transcription factor Snail mRNA expression in the CML blast crisis leukemic K562 cells exposured by vitamin E (100 µm) compared with metformin (4 mM) for 48 h cultured, calculated by 2- (ΔΔCt) method.

N n=3	Fold decreasing	Standard derivation, σ	Fold decreasing	Standard derivation, σ
	Vitamin E/ Snail M±m		Metformin / Snail M±m	
1	14,160 ± 0,437	0,408	1,579 ± 0,110	0,086
2	13,176± 0,547		1,366 ± 0,103	
3	13,833 ±0,110		1,464 ± 0,005	

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

From the analysis of the Table 1, according to the results of three independent studies (n = 3), we have detected the effect of vitamin E as specific to the repression of EMT phenotype through down-regulation of initial EMT-inducer transcription factor SNAIL in K562 CML blast crisis leukemic K562 cells. This is corresponded to ±SD value of 13,72 ($p<0.05$) fold-decreasing the expression of EMT-inducer Snail by vitamin E, in contrast to ±SD value 1,47 ($p<0.05$) fold-decreasing by metformin, respectively. It was recognised higher Snail inhibition effect by vitamin E compared with metformin on 9, 33 fold. Taken together, we have revealed that vitamin E is significantly more effective than metformin in Snail-EMT repression in the CML blast crisis leukemic cell line K562.

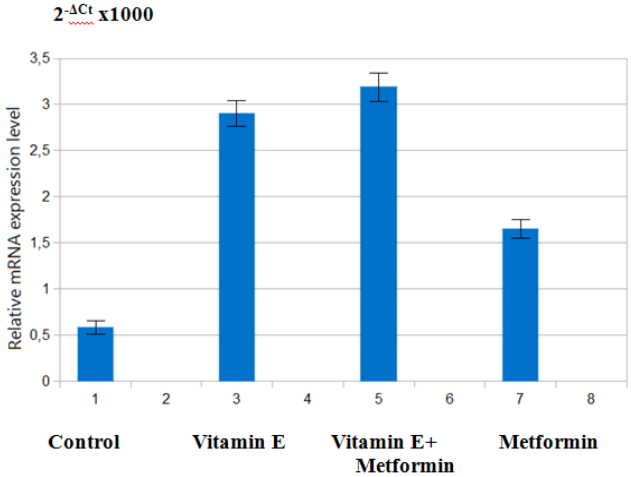

Figure 2. Relative mRNA expression level of transcription factor CEBPα as muster regulator myelopoiesis in CML blast crisis K562 cells and K562 cells exposured by vitamin E (100 µM) and metformin (4mM) for 48 houers, calculated by 2- (ΔCt) method. Control: K562; Vitamin E: K562 + vitamin E; Vitamin E+Metformin: K562 + vitamin E+ metformin; Metformin: K562 + metformin;

As see at Fig. 2, Control, the CEBPα myelopoietic muster regulator is crucial repressed in CML blast crisis K562 cells (±SD value 0,582), directly confirming its reverse dependence on Snail EMT-inducer overexpression in K562 cells (see Fig. 1, Control). The treatment CML blast crisis K562 cells by vitamin E (100 µM) for 48 h significant restores CEBPα mRNA expression in K562 cells ((±SD value 2,809, $p<0.001$) as was study by real-time RT-PCR . Metformin (4 mM) exposured K562 cells for 48 h was less efficient than vitamin E during CEBPα myelopoietic muster regulator re-expression in K562 cells (±SD value 1,652, $p<0.01$) whereas together with vitamin E treated K562 cells metformin observed a possible synergistic effect on vitamin E-dependent CEBP alpha gene expression induced potential in CML blast crisis K562 leukemic cells ((±SD value 3,191, $p<0.001$). Taken together, we have revealed that vitamin E is more effective in restored expression of CEBP alpha myeloid muster regulator in CML blast crisis K562cells then metformin.

Table 2. The Fold-increasing of relative levels of the transcription factor CEBP alpha mRNA expression in the CML blast crisis leukemic K562 cells exposured by vitamin E (100 µM) compared with metformin (4 mM) for 48 hours cultured, calculated by 2- (ΔΔCt) method.

N n=3	Fold increasing	Standard derivation, σ	Fold increasing	Standard derivation, σ	Fold increasing	Standard derivation, σ
	Vitamin E/ Snail M±m		Vitamin E+ Metformin M±m		Metformin M±m	
1	5,156 ± 0,328	0,232	5,564 ± 0,075	0,371	2,841±0,007	0,272
2	4,634± 0,194		5,00 ± 0,489		3,164±0,330	
3	4,696±0,132		5,902± 0,413		2,498± 0,336	

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

From the analysis of the Table 2, according to the results of three independent studies (n = 3), we also have suggested the detectable effect of vitamin E as specific to restorere the myeloid differentiation potential through up-regulation of myeloid muster regulator CEBPα mRNA expression in CML blast crisis leukemic K562 cells. This is corresponded to±SD value of 4,83 ($p<0.001$) fold-increasing the expression of CEBP alpha by vitamin E, in contrast to ±SD value 2,84 ($p<0.001$) fold-increasing by metformin, respectively. It was recognised higher CEBPα activation effect by vitamin E compared with metformin on 1, 70 fold-increasing. Taken together, we have revealed that vitamin E is more effective than metformin in restored CEBP alpha myelopoietic potential in the blast crisis CML cell line K562. Nevertheless, together with vitamin E exposure in K562 cells metformin showed little synergistic effect as ±SD value 5,49 ($p<0.001$) fold-increasing of CEBPα mRNA expression by metformin versus ±SD value 4,83 ($p<0.001$) fold-increasing of CEBPα mRNA expression by vitamin E itself.

The Table 3 and Table 4 are presented the Statistical significance of differences for three independent studies (n = 3) that was evaluated by Student's t-test for transcription factors Snail EMT-inducer and CEBP alpha myelopoietic master regulator investigation in CML blast crisis leukemic cell line K562 and modulated by vitamin E in comparison with metformin.

Table 3: The statistical significance (Student t-test) of Snail mRNA expression inhibition by vitamin E (100 µM) and metformin (4 mM) in culture K562 blast crisis of chronic myeloid leukemia (CML). Values of SYBR Green fluorescence optical density in qRT-PCR assay).

N n=3	Control	Vitamin E	t	P	Metformin	t	P
	M±m	M±m			M±m		
1	55,525 ±6,608	3,799 ±0,282	10,38	<0.001	35,158± 1,946	3,425	< 0,05
2	44,194 ±4,725	3,354 ±0,163			32,352± 0,86		
3	47,039 ±1,88	3,400 ±0,117			32,128 ± 1,084		
	M1 = 48,919	M2 = 3,517			M3 = 33,212		
	m1 = 4,404	m2 = 0,187			m3 = 1,296		

Note: $t = \frac{M1 - M2}{\sqrt{m1^2 + m2^2}}$ df = (n1 + n2) -2

Table 4: The statistical significance (Student t-test) of CEBP alpha mRNA expression increasing by vitamin E (100 µM) and metformin (4 mM) in culture K562 blast crisis of chronic myeloid leukemia (CML). Values of SYBR Green fluorescence optical density in qRT-PCR assay.

N n=3	Control	Vitamin E	Vitamin E+Metformin	Metformin
	M±m	M±m	M±m	M±m
1	0,574±0,008	2,960±0,151	3,194±0,003	1,631±0,02
2	0,596±0,014	2,762±0,047	2,980±0,211	1,886±0,234
3	0,576±0,006	2,705±0,104	3,400±0,209	1,439±0,213
	M1=0,582	M2=2,809	M3=3,191	M4=1,652
	m1=0,0093	m2=0,101	m3=0,141	m4=0,0243
		t=20,06	t=16,204	t=5,944
		P<0,001	P<0,001	P<0,01

Note: $t = \frac{M1 - M2}{\sqrt{m1^2 + m2^2}}$ df = (n1 + n2) -2

Taken together on the data obtained we observed that Snail1overexpression suggests the EMT phenotype in CML blast crisis progression and thereby contributes in CML pathogenesis. Also, we have suggested down-

regulation CEBP alpha transcription factor master regulator of myelopoiesis in CML blat crisis K562 cells as crucial event of Snail EMT-inducer phenotype. Our findings are consistent with the recent report by Lourenço A.N., et al. (2020) who suggest that C/EBP α is crucial determinant of epithelial maintenance by preventing epithelial-to-mesenchymal transition (EMT).[43] These authors stressed that C/EBP transcription factors play a pivotal role during terminal differentiation also of a variety of cells types and decreased levels of C/EBPα have been found also in diferent types of solid tumors.[43] Indeed, we have founded that CEBP alpha muster myeloid differentiation factor is repressed by overexpression of EMT-inducer Snail in CML blast crisis K562 cells. Consequently, re-activation of CEBP alpha reverse dependents from targeted suppression of Snail EMT-inducer as we detected by vitamin E effects in K562 cells in vitro. Our findings deepened the function of vitamin E and determined its anti-EMT role in CML blast crisis progression. Indeed, we observed that vitamin E was effective modulator of down-regulation of transcription factor Snail EMT-inducer and up-regulation of myelopoietic transcription factor CEBP alpha as result of vitamin E-dependent EMT repression in K562 cells. We concluded that vitamin E can be used as potential natural modulator of emerging EMT therapeutics in CML progression and restored the key CEBP alpha myeloid differentiation potential in imatinib-resistace CML leukemic cells taking into account the report by Kagita et al. (2015) demonstrating correlation of C/EBPα expression with response and resistance to imatinib in CML.[44]

CONCLUSION

Our results extend the role vitamin E beyond only differentiation-like factor in CEBP alpha induction in CML blast crisis K562 cells as we earlier elucidated.[31] The present results establish vitamin E role as novel regulator EMT suppression in CML blast crisis K562 cells and stressed reverse relationship between these both events. EMT-inducer Snail overexpression reverts myeloid CEBP alpha muster regulator expression in CML blast crisis K562 cells whereas vitamin E-dependent Snail1 supression returns CEBP alpha expression as we studied on the level of mRNA expression, respectively.

Conclusive key Points

1. These results confirm the importance of Snai1 EMT-inducer overexpression and repression of myeloid muster regulator CEBP alpha in CML blast crisis pathogenesios.
2. The present findings observed the tightly reverse relationship between transcription factor Snail as initial EMT-inducer and transcription factor CEBP alpha as myeloid muster regulator of normal hematopoietic stem cells (HSCs) in CML blast crisis leukemic K562 cells.
3. It first was revealed that vitamin E is effective modulator of down-regulation of EMT transcription factor Snail and up-regulation of myeloid differentiation transcription factor CEBP alpha in CML blast crisis K562 cells exposured.
4. Data obtained suggest that C/EBPα induction can function as suppressor CML blast crisis progression in K562 cells through vitamin E-dependent EMT-repression mechanism.
5. Conclusively, the vitamin E modulation role true argued causal relationship between EMT stemnes suppression and restored CEBP alpha myeloid differentiation potential in CML blast crisis cells.
6. The vitamin E action was more efficancy then metformin in both Snail-EMT repression and re-activation CEBP alpha in CML blast crisis K562 cells relatively exposured.
7. It is proposed that vitamin E (alpha-tocopherol) may be natural modulator EMT in LSC phenotype reprogramming in biotherapies of CML.

REFERENCES

1. Nieto M.A. Epithelial plasticity: a common theme in embryonic and cancer cells. Science, 2013; 342: 1234850.
2. Thiery J.P. Epithelial-mesenchymal transitions in tumour progression. Nature Reviews Cancer, 2002; 2: 442– 454.
3. Mani S.A., Guo W., Liao M-J. et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell, 2008; 133(4): 704-15.
4. Jolly M.K., Huang B., Lu M. et al. Towards elucidating the connection between epithelial mesenchymal transitions and stemness. J R Soc Interface, 2014; 11: 20140962
5. Singh A., Settleman J. EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. Oncogene, 2010; 29: 4741-4751.
6. Shibue T., Weinberg R.A. EMT, CSCs, and drug resistance: the mechanistic link and clinical implications. Nature Reviews Clinical Oncology, 2017; 4: 611-629.
7. Voulgari A., Pintzas A. Epithelial-mesenchymal transition in cancer metastasis: mechanisms, markers and strategies to overcome drug resistance in the clinic. Biochimica et Biophysica Acta, 2009; 1796: 75– 90.
8. Du B., Shim J. Targeting epithelial–mesenchymal transition (EMT) to overcome drug resistance in cancer. Molecules, 2016; 21: 965.
9. Santamaria P., Moreno-Bueno G., Cano A. Contribution of Epithelial Plasticity to Therapy Resistance. Journal of Clinical Medicine, 2019; 8(5): 676.
10. Nieto MA, Cano A. The epithelial-mesenchymal transition under control: global programs to regulate epithelial plasticity. Semin Cancer Biol, 2012; 22: 361–8.
11. Lamouille S., Xu J., Derynck R. Molecular mechanisms of epithelial–mesenchymal transition. Nat Rev Mol Cell Biol, 2014;15:178–196.

12. Dang H., Ding W., Emerson D., Rountree C.B. Snail1 induces epithelial-to-mesenchymal transition and tumor initiating stem cell characteristics. BMC Cancer, 2011; 11: 396.
13. Wang Y., Shi J., Chai K. et al. The Role of Snail in EMT and Tumorigenesis . Curr Cancer Drug Targets, 2013; 13(9): 963–972.
14. Cano A., Perez-Moreno M.A, Rodrigo I. et al. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat Cell Biol, 2000; 2: 76–83.
15. Nieto MA. The Snail superfamily of zinc-finger transcription factors. Nat Rev Mol Cell Biol, 2002; 3: 155–166.
16. Olmeda D., Jorda M., Peinado H., Fabra A., Cano A. Snail silencing effectively suppresses tumour growth and invasiveness. Oncogene, 2007; 26: 1862–1874.
17. Chen S., Liao T., Yang M. Emerging roles of epithelial-mesenchymal transition in hematological malignancies. J Biomed Sci, 2018; 25: 37.
18. Kidan N., H., Ruimi N., Roitman Sh. Ectopic Expression of Snail and Twist in Ph+ Leukemia Cells Upregulates CD44 Expression and Alters Their Differentiation Potential. Journal of Cancer, 2017; 8(8): 3952-3968.
19. Carmichael C.L., Haigh J.J. The Snail Family in Normal and Malignant Haematopoiesis.
20. Cells Tissues Organs, 2017; 203:82-98
21. Carmichel C.L., Goossens S., Wang J. et al. The EMT Modulator SNAI1 Drives AML Development Via Its Interaction with the Chromatin Modulator LSD1. Blood, 2016; 128 (22): 2688.
22. Sayyler V., Griffin J.D. Molecular mechanisms of transformation by the BCR-ABL oncogene. Semin Hematol, 2003; 40: 4–10.
23. Calabretta B., Perrotti D. The biology of CML blast crisis. Blood, 2004; 103: 4010–22.
24. Zhou H., Xu R. Leukemia stem cells: the root of chronic myeloid leukemia. Protein & Cell, 2015; 6: 403-412.
25. Crews L.A., Jamiesson C.H.M. Chronic Myeloid Leukemia Stem Cell Biology. Curr Hematol Malig Rep, 2012; 7(2): 125-132.
26. Morotti A., Panuzzo C., Fava C., Saglio G. Kinase-inhibitor-insensitive cancer stem cells in chronic myeloid leukemia. Expert Opin Biol Ther, 2014; 14: 287–99.
27. PuissantA., Dufies M., Fenouille N. et al. Imatinib triggers mesenchymal-like conversion of CML cells associated with increased aggressiveness. Journal of Molecular Cell Biology, 2012; 4(4): 207–220.
28. Soverini S., Mancini M., Bavaro L. et al. Chronic myeloid leukemia: the paradigm of targeting oncogenic tyrosine kinase signaling and counteracting resistance for successful cancer therapy. Mol Cancer, 2018; 17: 49.
29. Hanekamp D., Cloos J., Schuurhuis G.J. Leukemic stem cells: identification and clinical application. Int J Hematol, 2017; 105: 549–557.
30. Testa U. Leukemia Stem Cells. Ann Hematol, 2011; 90(3): 245-71.
31. Shvachko L.P. EMT mechanism in stemnes phenotype induction in myeloproliferative leukemias. Factors of experimental evolution of organisms, 2018; 23: 256-270. (Ukrainian).
32. Shvachko L.P., Zavelevich M.P., Gluzman D.F., Kravchuk I.V., Telegeev G.D. Vitamin E activates expression of C/EBP alpha transcription factor and G-CSF receptor in leukemic K562 cells. Exp Oncol, 2018; 40(4): 328–331.
33. Jamieson C., Ailles L., Della S., et al. Granulocyte–Macrophage Progenitors as Candidate Leukemic Stem Cells in Blast-Crisis CML. N Engl J Med, 2004; 351: 657-667.
34. Wang Q.F., Friedman A.D. CCAAT/enhancer-binding proteins are required for granulopoiesis independent of their induction of the granulocyte colony-stimulating factor receptor. Blood, 2002; 99: 2776–85.
35. Porse B.T, Bryder D., Theilgaard-Monch K. et al. Loss of C/EBP alpha cell cycle control increases myeloid progenitor proliferation and transforms the neutrophil granulocyte lineage.JExpMed.2005;202:85–96.

    35. Avellino R., Delvel R.. Expression and regulation of C/EBPα in normal myelopoiesis and in malignant transformation. Blood, 2017; 129: 2083–2091.
36. Paz-Priel I., Friedman A.D. C/EBPα Dysregulation in AML and ALL. Crit Rev Oncog, 2012; 16: 93–102.
37. Pullikan J.A., Tenen D.G., Behre G. C/EBPα deregulation as a paradigm for leukemogenesis. Leukemia, 2017; 31: 2279–85.
38. Tavor S., Park D.J., Gery S., et al. Restoration of C/EBPalpha expression in a BCR- BL^{+}Cell line induces terminal granulocytic differentiation. J Biol Chem, 2003; 278: 52651–52659.
39. Nieborowska-Skorska M., Hoser G., Hochhaus A. et al. Anti-oxidant vitamin E prevents accumulation of imatinib-resistant BCR-ABL1 kinase mutations in CML-CP xenografts in NSG mice. Leukemia, 2013, 27(11): 2253–2254.
40. Benerjee P., Surendrun H., Chowdhury D.R. et al. Metformin mediated reversal of epithelial to mesenchymal transition is triggered by epigenetic changes in E-cadherin promoter. J Mol Med (Berl), 2016; 94(12): 1397-1409.
41. Cufi S., Vazquez-Martin A., Oliveras-Ferraros C. et al. Metformin against TGFβ-induced epithelial-to-mesenchymal transition (EMT): from cancer stem cells to aging-associated fibrosis. Cell Cycle, 2010; 9(22): 4461-4468.
42. Schmittgen Th.D, Livak K.J. Analyzing real-time PCR data by the comparative Ct method. Nature Protocols, 2008; 3: 1101-8.
43. Lourenço A.N., Roukens M.G., Seinstra D. et al. C/EBP α is crucial determinant of epithelial maintenance by preventing epithelial-to-

mesenchymal transition. Nature Communication, 2020; 11: 785.
44. Kagita S., Uppalapati S., Gungeti S., Digumarti R. Correlation of C/EBPα expression with response and resistance to imatinib in chronic myeloid leukemia. Jpn J Clin Oncol, 2015; 45: 749–54.