The evaluation of the TGF- β1 and TβRII gene expression in patients with acute lymphoblastic leukemia

Hamideh Aghaeenezhad (1)
Mohammad Hossein Mohammadi (2)
Gholamreza Toogeh (3)
Sina Salari (4)
Mohammadreza Reza Khosravi (5)
Fatemeh Mezginezhad (6)
Mehdi Allahbakhshian-Farsani (7)

(1) Hamideh Aghaeenezhad (MSc), MSc in Laboratory Hematology and blood Banking, Laboratory Hematology and blood Banking Department, School of Allied medical sciences, Shahid Beheshti University of Medical Science, Tehran, Iran
(2) Assistant Professor in Laboratory Hematology and blood Banking (Lab Advisor) Laboratory Hematology and Blood Bank Department, Faculty of Paramedical, Shahid Beheshti University of Medical Sciences, HSCT Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
(3) Professor of Medical Oncology and Hematology in adults (Clinical Advisor) Thrombosis and Homeostasis Research Center, Imam Khomeni Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran.
(4) Assistant Professor of Medical Oncology, Hematology and Bone Marrow Transplantation, Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran HSCT Research Center, Shahid Beheshti University of medical sciences, Tehran, Iran
(5) Assistant Professor of Medical Oncology and Hematology in adults Cancer prevention research center, Isfahan University of Medical Science, Isfahan, Iran
(6) MSc in Laboratory Hematology and blood Banking Laboratory Hematology and blood Banking Department, School of Allied medical sciences, Shahid Beheshti University of Medical Science, Tehran, Iran
7. Mehdi Allahbakhshian-Farsani (PhD), Assistant Professor in Laboratory Hematology and blood Banking, Laboratory Hematology and Blood Bank Department, Faculty of Paramedical, Shahid Beheshti University of Medical Sciences, HSCT Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Corresponding author:
Mehdi Allahbakhshian-Farsani
Laboratory Hematology and Blood Bank Department, Faculty of Paramedical,
Shahid Beheshti University of Medical Sciences, HSCT Research Center, Shahid Beheshti University of Medical Sciences,
Tehran, Iran
Email: allahbakhshian@sbmu.ac.ir

Abstract

Background: Tumor suppressors are key molecules involved in the malignant process. TGF- β is one of the most important suppressor genes with a complex role in intracellular processes. Although TGF-β is a traditional tumor suppressor; recent evidence has shown its promoter role in solid tumors. However, it is not determined whether TGF-β has a tumor suppressor or tumor promoter role in hematologic malignancies. In this study we evaluated the expression of TGF-β and its receptor in patients with acute lymphoblastic leukemia, as an important hematologic malignancy.

Material and Method: In this study, the expression of TGF-β and TβRII was analyzed in 52 patients with acute lymphoblastic leukemia in comparison with 13 normal controls; all of them informed volunteers. The mononuclear cell was separated using Ficoll for RNA isolation. After synthesis of c-DNA, the gene expression was measured using cyber green RQ-PCR.

Results: Our results showed that the expression level of TGF-β (3.6 fold) and TβRII (7.7 fold) was significantly decreased in all patient groups in comparison with healthy controls. Reduction in TGF-β was significantly correlated to blast count; TGF-BRII reduction also had correlation with chromosomal translocation, however, we did not observe any correlation with other parameters such as age, gender and leukemic cell immunophenotype.

Conclusion: Altogether our findings suggest defeated TGF-β signaling in patients with acute lymphoblastic leukemia (ALL), and it seems that the targeting of TGF- β signaling component is one of the basic and essential mechanisms in cancer development. More research in this field can help us to design novel methods for ALL diagnosis, classification, monitoring and treatment.

Key words: acute lymphoblastic leukemia, gene, TGF- β , TGF- BRII

Please cite this article as: Mehdi Allahbakhshian-Farsani et al. The evaluation of the TGF- β1 and TβRII gene expression in patients with acute lymphoblastic leukemia. World Family Medicine. 2018; 16(2): 198-206. DOI: 10.5742/MEWFM.2018.93262

Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disorder of hematopoietic stem cells which is characterized by accumulation of immature and inefficient lymphoid precursors (lymphoblasts) in bone marrow and other lymphoid organs (1). Generally the risk classification of ALL patients is based on patient's age, primary WBC count, disease's immunephenotype, chromosomal abnormalities and treatment response rate (2). All of the above prognostic markers are highly dependent on substantial molecular defects of the leukemic cells. Routinely, prognostic categorization of patients based on the molecular features assists physicians greatly in choosing more successful treatment strategies (3).

Tumor suppressors and oncogenes are two types of key molecules involved in the malignant process (4,12). Oncogenes have traditionally attracted more attention in the scientific research. However, recent studies clearly proved the essential role of tumor suppressor defects in cancer development (13,18). In this regard, TGF-β signaling has been reported as a prominent tumor suppressor by several studies (19-21). TGF-β regulates the expression of a wide range of genes involved in critical cellular functions including cell cycle, cell differentiation, cell apoptosis, hematopoietic stem cell dormancy, extracellular matrix formation, genetic integrity and cell migration (22,27).

Naturally, TGF- β induces the expression of cell cycle inhibitors (such as P15, P21 and P53) and inhibits cell cycle inducers (such as c-myc and ID family of proteins) thereby it inhibits tumor formation and induces cell differentiation at the same time(28). Different studies indicated TGF-β signaling abnormalities in tumors. In this way, the aberrant expression of TGF- β has been reported in different kinds of malignancies such as colon cancer, breast cancer and hematological neoplasms (29-32). Other studies demonstrated mutation in other elements of TGF-β signaling pathway including TβRII and SMAD proteins in human malignancies (33,34). In hematologic malignancies it has also been demonstrated that some fusion genes such as AML1-EVI1 and TEL-AML1 inhibits TGF-β signaling pathway elements. All these studies proved tumor suppressor effects of TGF-β signaling (35-37).

Although, these studies indicated tumor suppressor role for TGF-β, recent studies unexpectedly showed tumor promoter function for this signaling pathway in some types of cancers which indicates context dependent function of TGF-β in different kinds of malignancies(32). This complexity is greatly dependent on factors such as the type of malignancy, tumor environment, types of genetic abnormalities, stage of malignancy and the rate of tumor progression (38-40). The tumor promoter function of TGF-β has been mainly explained by alternative signaling. In normal tissues, classic TGF-β signaling occurs through SMAD pathway of proteins. TGF-β signaling by SMAD activates the transcription of several target genes with tumor suppressor activity(41). However, during alternative TGF-β signaling in tumor promoter context, TGF-β signaling doesn't occur through SMADs pathway any more. In this condition, alternative signaling by MAP kinases, PI3K /AKT, GTPase Rho-like leads to malignant cells proliferation and survival (42,43).

Acute lymphoblastic leukemia (ALL) is a malignant disorder of hematopoietic stem cells which is characterized by accumulation of immature and inefficient lymphoid precursors (lymphoblasts) in bone marrow and other lymphoid organs (1). Generally the risk classification of ALL patients is based on patient's age, primary WBC count, disease's immunephenotype, chromosomal abnormalities and treatment response rate (2). All of the above prognostic markers are highly dependent on substantial molecular defects of the leukemic cells. Routinely, prognostic categorization of patients based on the molecular features assists physicians greatly in choosing more successful treatment strategies (3).

Tumor suppressors and oncogenes are two types of key molecules involved in the malignant process (4,12). Oncogenes have traditionally attracted more attention in the scientific research. However, recent studies clearly proved the essential role of tumor suppressor defects in cancer development (13,18). In this regard, TGF-β signaling has been reported as a prominent tumor suppressor by several studies (19-21). TGF-β regulates the expression of a wide range of genes involved in critical cellular functions including cell cycle, cell differentiation, cell apoptosis, hematopoietic stem cell dormancy, extracellular matrix formation, genetic integrity and cell migration (22,27).

Naturally, TGF- β induces the expression of cell cycle inhibitors (such as P15, P21 and P53) and inhibits cell cycle inducers (such as c-myc and ID family of proteins) thereby it inhibits tumor formation and induces cell differentiation at the same time(28). Different studies indicated TGF-β signaling abnormalities in tumors. In this way, the aberrant expression of TGF- β has been reported in different kinds of malignancies such as colon cancer, breast cancer and hematological neoplasms (29-32). Other studies demonstrated mutation in other elements of TGF-β signaling pathway including TβRII and SMAD proteins in human malignancies (33,34). In hematologic malignancies it has also been demonstrated that some fusion genes such as AML1-EVI1 and TEL-AML1 inhibits TGF-β signaling pathway elements. All these studies proved tumor suppressor effects of TGF-β signaling (35-37).

Although, these studies indicated tumor suppressor role for TGF-β, recent studies unexpectedly showed tumor promoter function for this signaling pathway in some types of cancers which indicates context dependent function of TGF-β in different kinds of malignancies(32). This complexity is greatly dependent on factors such as the type of malignancy, tumor environment, types of genetic abnormalities, stage of malignancy and the rate of tumor progression (38-40). The tumor promoter function of TGF-β has been mainly explained by alternative signaling. In normal tissues, classic TGF-β signaling occurs through SMAD pathway of proteins. TGF-β signaling by SMAD activates the transcription of several target genes with tumor suppressor activity(41). However, during alternative TGF-β signaling in tumor promoter context, TGF-β signaling doesn't occur through SMADs pathway any more. In this condition, alternative signaling by MAP kinases, PI3K /AKT, GTPase Rho-like leads to malignant cells proliferation and survival (42,43).

TGF- β has an essential role in the functional regulation of blood cells such as monocytes, macrophages, neutrophils, platelets and even hematopoietic stem cells; moreover, these cells are also the essential sources of TGF- β secretion (44). However, most studies on the role of TGF-β signaling have been performed on solid tumors and the role of TGF- β signaling in hematologic malignancies has been less considered. This study attempts to evaluate the expression of TGF- β and its receptor (TβRII) in acute lymphoblastic leukemia patients to better clarify the possible role of this cytokine in malignant lymphoblasts.

Methods

Patient samples

The present study was performed on the bone marrow (BM) and peripheral blood (PB) samples obtained from newly diagnosed ALL patients and 13 normal control subjects, with informed consent. Consent letter was approved by the local Ethics Committee. The patients were referred to Mofid, Taleghani and Emam Khomeini Hospital, Tehran, Iran with diagnoses based on clinical features and laboratory tests including: morphological assessment, immunophenotyping (according to the FAB classification system) and molecular studies. The median age of individuals in this study was 18 years, with a range of 1–89 years and mean age of 26 years. Samples were taken from 22 female and 30 male subjects.

RNA isolation, cDNA synthesis, quantitative realtime PCR

Total cellular RNA was extracted from MNCs using RNeasy minikit (Qiagen, Germany). The quality and quantity of extracted RNA was measured by NanoDrop (Thermo Scientific, Wilmington, North Carolina, USA) (OD 260/280 nm ratio >1.8). Subsequently, 2 µL (0.5 mg) RNA was used for cDNA synthesize in a final volume of 20 µL using a Thermo Scientific kit (Qiagen, Hudson, NH, USA). c-DNA synthesis was checked by ABL primer as housekeeping gene. An aliquot of 1/10th of the resulting cDNA from control and patient (1 µL) was used as substrate for qRT- PCR amplification. Primers specific to TGF- β1 and TβRII and ABL1 (housekeeping gene) were designed using oligo7 software [Table 1] using data obtained from NCBI databases and designed primers were evaluated for specificity by NCBI primer BLAST. Consequently, the expression of TGF- β1 and TβRII and ABL1 mRNA was analyzed by qRT-PCR (Rotor Gene 6000, Bosch, Qiagen, Germany). qRTPCR reaction components for each gene were composed of 1 µL of template cDNA, 1 µL primer(forward and reverse), 7 µL of RealQ Plus 2x Master Mix GreenLow ROX (Ampliqon, Denmark), and 6 µL water for a total reaction volume of 15 µL. For each qRTPCR reaction, standard curve was considered using five consecutive 1:10 dilutions of cDNA sample (1, 0.1, 0.01, and 0.001). The thermal cycler conditions for each reaction(TGF- β1 and TβRII) consist of initial holding at 95°C for 10 minutes, second phase as denaturation at 95°C for 10 s including 40 cycles, annealing/extension at

65°C for 15 s and final extension at 72°C for 10 minutes. All experiments were performed in duplicate and the relative quantification of mRNA expression for each sample (fold change = FQ) was calculated using the Livak method (2-ΔΔct). (Livak & Schmittgen, 2001; Schmittgen & Livak, 2008).

Statistical Analysis

Data analysis was performed using the SPSS Statistics (V 16.0) and GraphPad Prism (V 6.07) software. The results were represented in form of Mean+SEM. Shapiro Wilk and the Kolmogorov-Smirnov tests were used to evaluate the normal distribution of TGF-β1 and TβRII expression in ALL patients and control group. The t-test was also used to determine whether there was a significant difference in TGF- β1 and TβRII expression between ALL patients and the normal controls. The Pearson's chi-squared test was used to analyze the correlation between TGF- β1 and TβRII expression. The ANOVA test was applied to evaluate the differential expression of TGF- β1 and TβRII according to FAB classification. (p = 0.05 was considered as significance level).

Table 1: The sequence of forward and reverse primers for TGF-β genes, TGF-βRII and ABL1 gene is shown with the length of each primer with unit of nucleotide.

Gene	Premier	Sequence	Length
TGF-β	Forward	AAGGACCTCGGCTGGAAGTG	bp 20
	Reverse	CCCGGGCCATGCTGGTTGTA	bp 20
TGF-βRII	Forward	GGTTTTCAGTTATCTCCAGTCCA	23 bp
	Reverse	GGGGTCCAGGTAGGCAGTG	19 bp
ABL1	Forward	AGTCTCAGGATGCAGGTGCT	20 bp
	Reverse	TAGGCTGGGGCTTTTTGTAA	20 bp

Results

The expression rate of TGF-β and TGF-βRII in case and control groups:

The results showed that the expression of TGF-β (1.19 ± 0.07) and TBRII (1.01± 0.16) in ALL patients was significantly lower compared to normal controls with P value < 0.0015 (mean TGF-β in patients: 0.63 ± 0.08 and mean TGF- βRII in patients: 1.01 ± 0.16). TGF-β expression was 3.6 fold and TBRII expression was 7.7 fold lower than normal controls. There was no significant difference between gene expression levels of TGF-β and TGF-βRII in men and women (P value = 0.69 and 0.62 respectively). The patients were divided into two age classes of younger than 16 and older than 16 years. The expression rate of genes in the two age groups were evaluated, the results showed that there was no significant difference between the two age groups (P value =0.58 and 0.24)

The results showed that the expression of TGF-β (1.19 ± 0.07) and TBRII (1.01± 0.16) in ALL patients was significantly lower compared to normal controls with P value < 0.0015 (mean TGF-β in patients: 0.63 ± 0.08 and mean TGF- βRII in patients: 1.01 ± 0.16).

The expression rate of TGF- β and T BRII in case groups regarding to, translocation and immunophenotype classification:

Regarding existence of chromosomal translocation, patients were divided into two groups (trans positive and trans negative); our results showed no significant changes in TGF-β and Tβ RII expression between these groups(P value= 0.34 and 0.28). On the other hand, TGF-β and Tβ RII expression level was evaluated according to type of translocation including t(12:21), t(9:22), t(1:19) and t(4:11). The results showed that the expression level of TGF-β was not significantly different among patients with t(9:22), t(1:19) and t(4:11) translocations (P value=0.92), while the expression rate of TβRII was significantly higher in patients with t(12:21) (P value=0.04) in comparison with other translocations. Our patients were also categorized into B lineage and T lineage ALL. There was no significant difference between TGF-β and Tβ RII expression in these two groups. Patients were also categorized into 5 groups including T-ALL, Pro B ALL, Early pre B-ALL, Pre B-ALL and B-ALL. The expression level of TGF-β and TβRII was evaluated and the results showed no significant difference in TGF-β and TβRII expression level between different leukemic phenotypes (P value= 0.18 and 0.41)

Figure 1: The expression level of TGF-β and TGF-βRII in case and control groups

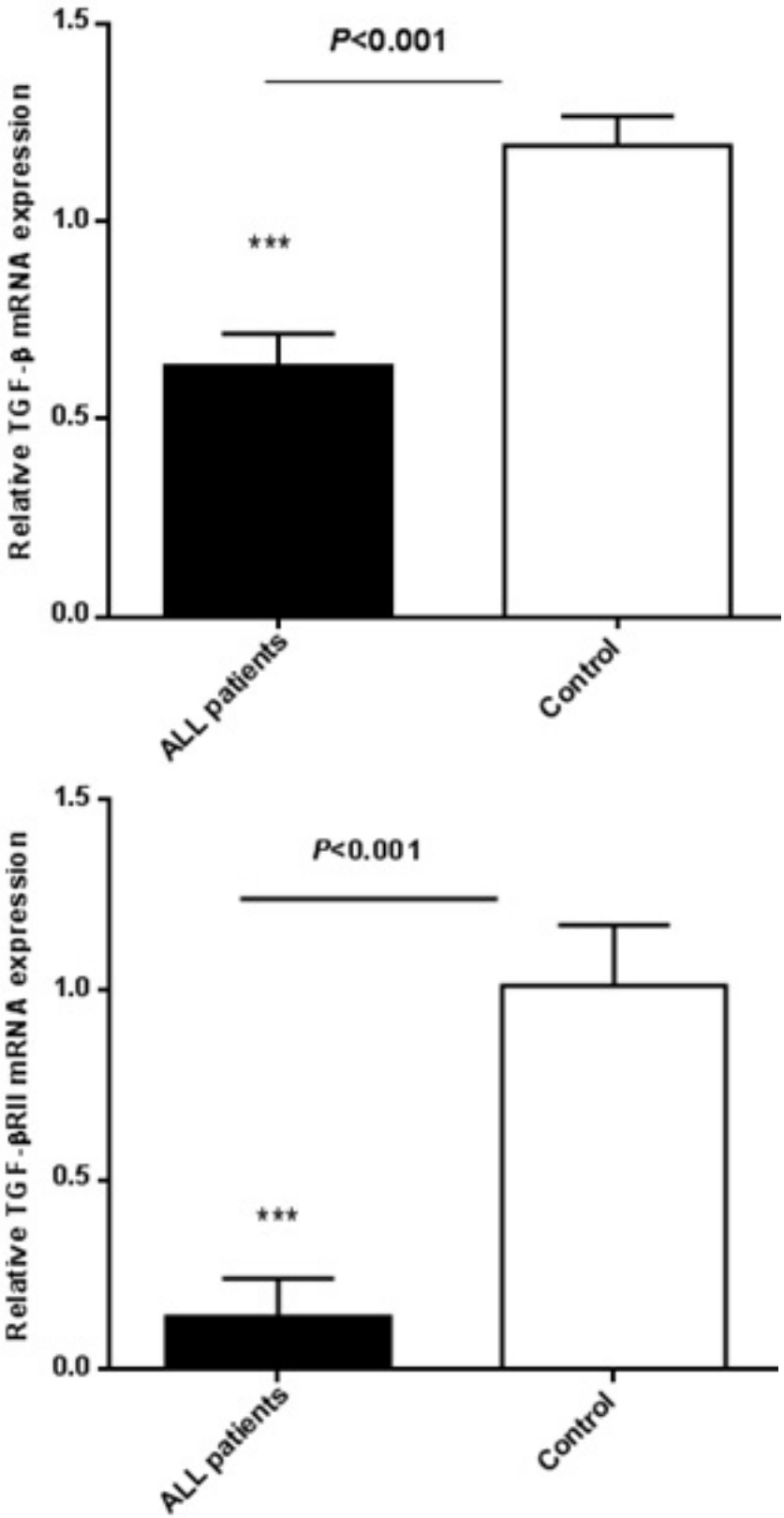
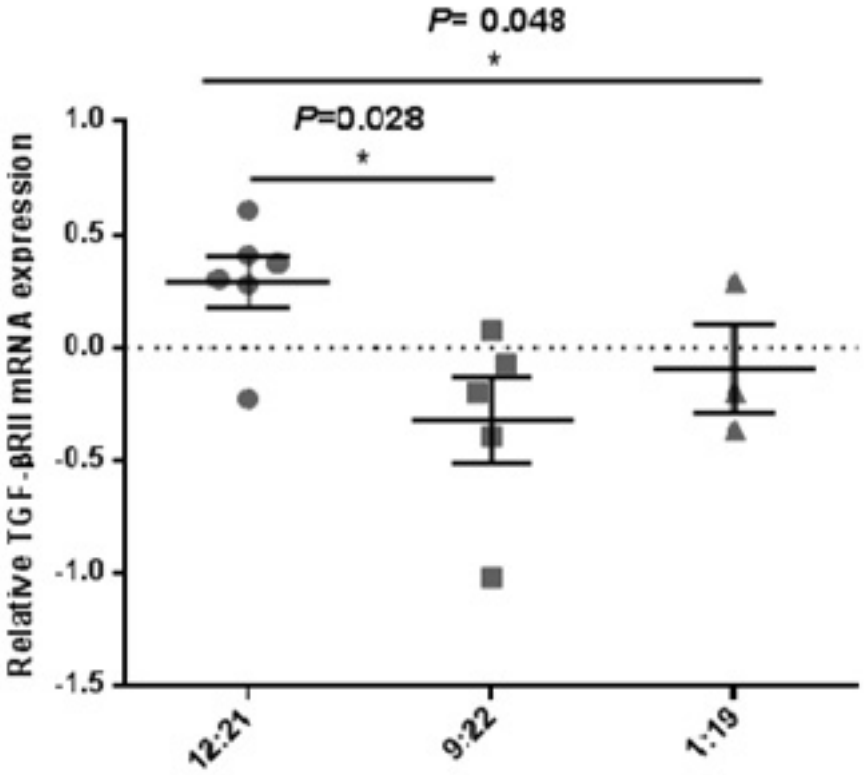


Figure 2: The expression level of TβRII in case groups regarding, translocation and immunophenotype classification



The expression rate of TβRII was significantly higher in patients with t(12:21) (P value=0.04) in comparison with other translocations. Our patients were also categorized into B lineage and T lineage ALL.

The correlation between the expression rate of TGF- β and TβRII:

Our results showed a positive and significant correlation between TGF- β and TβRII expression (p value= 0.015, r=0.33) (Chart 1) in our patients. Also there was a significant correlation between blast count and the expression rate of TGF- β and TβRII (P value=0.043, r=-0.288 and p value 0.74, respectively) (Chart 2), however there was no significant correlation between TGF- β and TβRII expression with age of patients (p value=0.15, r=0.293 and p value=0.089, r=0.293, respectively).

Chart 1: regression between TGF-β with TβRII

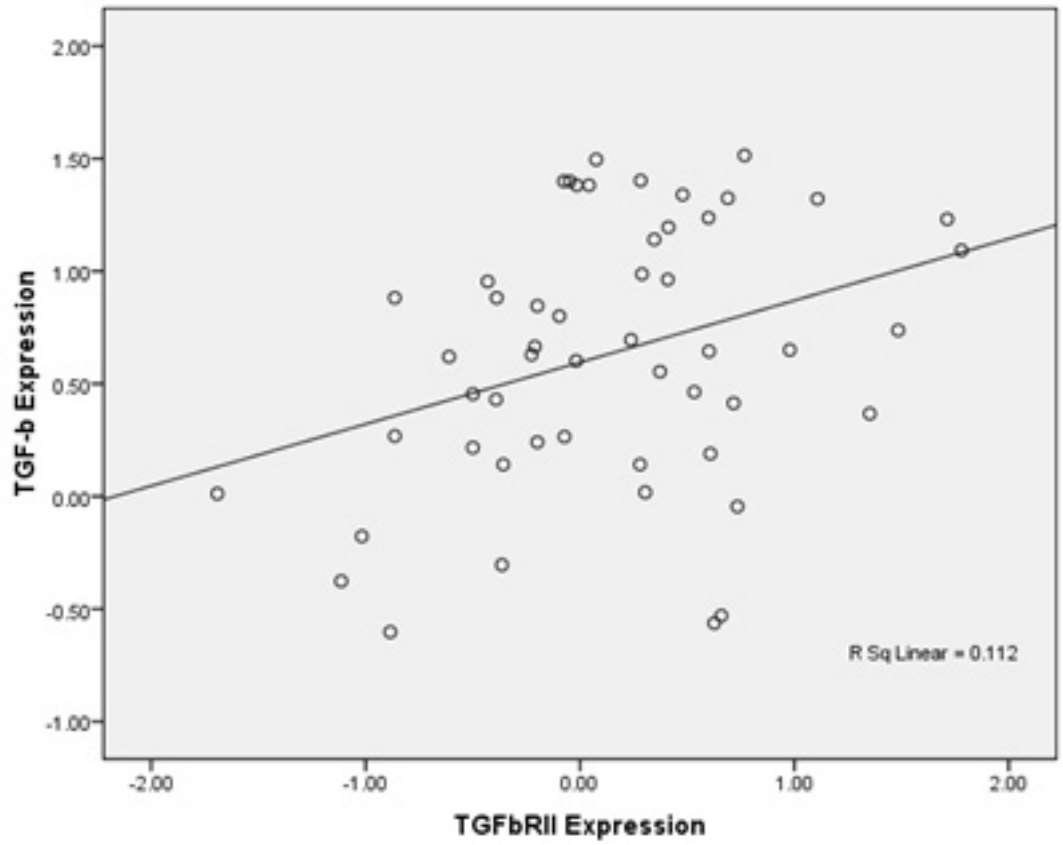
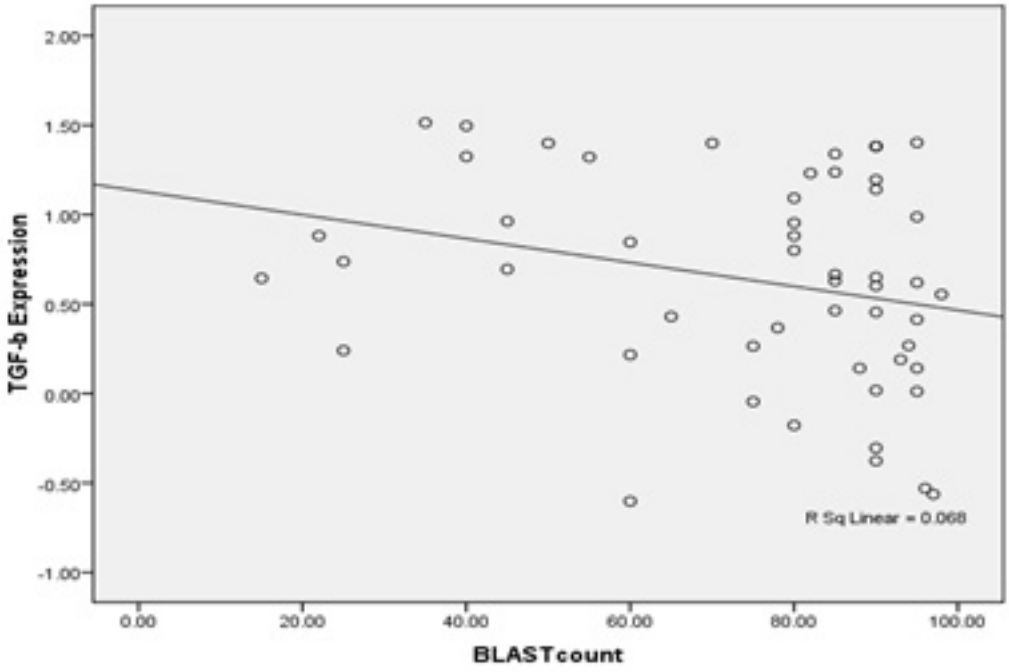


Chart 2: regression between TGF-β with blast count



Discussion and Conclusion

Despite all advances in deciphering basic mechanisms involved in ALL leukemogenesis, still ambiguities exist in relation to tumor formation and development (45). While proliferation inducing factors work unrestrainedly in cancer cells, tumor suppressors generally stop their function. Albeit, tumor suppressor defects have an essential role in tumorogenesis, they have attracted less attention (46). There are several potent tumor suppressors, among them TGF-β is one of the most important (47-49). However recent studies have shown that there is also a tumor promoter function for TGF-β signaling in some neoplasms as well (50), thus we decided to evaluate the expression of TGF-β and TβRII as two key molecules involved in TGF-β signaling, in patients with ALL. Our data demonstrated a significant reduction in the expression of TGF-β (3.6 fold) and TβRII (7.7 fold) in our patients compared with normal controls and most cases represented a simultaneous reduction in TGF-β and TβRII expression. Statistical analysis using Pearson test indicated a positive and significant correlation between TGF-β and TβRII expression. On the other hand, we did not observe any correlation in TGF-β or TβRII expression with clinical data such as age, gender and disease immunophenotype. These data suggest that TGF-β signaling may be a defected pathway in ALL patients and malignant cells inactivate TGF-β signaling to escape from anti-tumor effects of this pathway. Such failures in TGF-β signaling can remove negative pressure of TGF-β signaling on the cell cycle and permits neoplastic cells to enter further and faster into the proliferation phase (46, 48). In agreement with our findings, Renald's showed reduced TβRII expression in patients with T cell sezary syndrome (51). Moreover, Swati Biswas et al also demonstrated that TβRII mutations disabled TGF-β signaling and is an important factor in the initiation and progression of colon cancer (52). Nikolaos Soulitzi et al and Dos Reis et al proved that patients with prostatic cancer have reduced TGF-β expression as well (53,54). The involvement of other TGF-β signaling elements such as SMADs proteins has been observed in other studies, for example, Lin et al have shown that PML-RARα fusion gene inhibits the activation of SMAD2/3 and prevents TGF-β signaling to the nucleus. After ATRA therapy PML-RARα will be degraded and malignant cells respond to TGF-β signaling by their differentiation (55). Jakubowiak et al's study showed that AM1-ETO , another fusion gene in AML, also prevent TGF-β signaling through SMAD3 inhibition (56). A significant reduction in smad3 expression was also reported by Lawwrence et al in T ALL patients (57). All these studies along with our observation strongly suggest tumor suppressor role for TGF-β signaling and TGF-β signaling is inactivated as a possible mechanism for tumor escape from regulatory pressure. Although the natural role of TGF-β pathway indicates tumor suppressor activities, recent studies unexpectedly reported its role in tumor promotion as well; in this regard, Yong Wu, et al study represented over-expression of TβRII in acute myeloid leukemia. In these cases, over-expression of aberrant isoforms of TGF- βRII inhibits normal TGF-β signaling in patients with AML (58). Hui-Jun Zhang et al reported epithelial-mesenchymal transition (EMT) which is a feature of advanced stage of cancer, during TGF-β over-expression in lung carcinoma (59). Mele et al in their study showed the role of this cytokine in colon cancer metastasis also (60). Although in normal condition TGF-β stops cell cycle at G1 stage to inhibit cell proliferation and induces cell differentiation simultaneously. In the mentioned cases, TGF-β over-expression was unable to prevent disease initiation but it unexpectedly acts along with tumor promoter factors. However we did not observe such a finding in ALL patients.

In conclusion; according to our evaluation, TGF-β seems to act as a tumor suppressor in ALL patients because it showed lower expression in ALL patients in comparison with normal controls. It is possible that leukemic cells use TGF-β signaling down regulation for ease in their growth

and survival. Actually, TGF-β acts as a double edged sword in malignancies by contradictory signaling through alternative and classic pathways. The reason for the mentioned contradiction probably lies in natural differences in various tumors context and remarkable genetic and epigenetic heterogeneity between different kinds of malignancies. In this study, although it was not unexpected to see a significant difference in TGF-β expression between adults and children, between male and females and finally between different ALL subtypes, there was not any significant differences. It suggests TGF-β signaling deficiency may act as a general mechanism in tumor formation and promotion in ALL patients. Since simultaneous decreased expression level in both TGF-β and TβRII was seen, it is supposed that tumoral cells benefit from deactivation of multiple target genes in this pathway. Finally, due to the critical role of TGF-β pathway in cell regulatory mechanisms, the evaluation of the significance of TGF-β signaling elements in disease risk stratification, choosing therapeutic options and patient monitoring is highly recommended in future work.

References

1. Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. The Lancet. 2008 Mar 28;371(9617):1030-43.
2. Swerdllow S, Campo E, Harris NL. WHO classification of tumours of haematopoietic and lymphoid tissues: France: IARC Press, 2008; 2008.
3. Rowe JM. Prognostic factors in adult acute lymphoblastic leukaemia. British journal of haematology. 2010 Aug 1;150(4):389-405.
4. Knudson AG. Two genetic hits (more or less) to cancer Nature Reviews Cancer. 2001 Nov 1;1(2):157-62.
5. Emily Guo X, Ngo B, Sandaldjian Modrek A, Lee WH. Targeting tumor suppressor networks for cancer therapeutics. Current drug targets. 2014 Jan 1;15(1):2-16.
6. Salarpour F, Goudarzipour K, Mohammadi MH, Ahmadzadeh A, Faraahi S, Farsani MA. Evaluation of CCAAT/Enhancer Binding Protein (C/EBP) Alpha (CEBPA) and Runt-Related Transcription Factor 1 (RUNX1) Expression in Patients with De Novo Acute Myeloid Leukemia. Annals of Human Genetics. 2017 Nov 1;81(6):276-83.
7. Goudarzipour K, Ahmadzadeh A, Mohammadi MH. Changes of AML 1 and P53 tumor suppressor gene expression in patients de novo acute myeloid leukemia. Journal of Paramedical Sciences. 2017 Jan 17;8(1):39-45.
8. Mahmoudian-Sani MR, Mehri-Ghahfarrokhi A, Shojaeian A, Asadi-Samani M, Luther T. The role of microRNAs in human cancers. Immunopathol Persa. 2018;4(1):e05.
9. Dehghan Shahreza F. From oxidative stress to endothelial cell dysfunction. J Prev Epidemiol. 2016; 1(1):e04.
10. Mahmoudian Sani MR, Asadi-Samani M, Shirzad H, Moradi MT, Tamadon MR. Biomarkers in cancer. Persian J Front Cancers. 2018;1(1):e03.
11. Nasri P. Mitochondria as a biomarker for cancer therapy. Aria J Front Biomark. 2018;1(1):e01.
12. Salami A, Amiri M. On the occasion of world cancer day 2017; breast cancer. Journal of Ischemia and Tissue Repair. 2017;1(1):e02.
13. Wenzl K, Troppan K, Neumeister P, Deutsch AJ1. The nuclear orphan receptor NR4A1 and NR4A3 as tumor suppressors in hematologic neoplasms. Curr Drug Targets. 2015;16(1):38-46.
14. Mercher T, Quivoron C, Couronné L, Bastard C, Vainchenker W, Bernard OA. TET2, a tumor suppressor in hematological disorders. Biochim Biophys Acta.2012 Apr;1825(2):173-7. doi: 10.1016/j.bbcan.2011.12.002. Epub 2012 Jan 3.
15. Taghipour M, Motalebi M, Einollahi B, Moradi MZ, Sarhangi A. A dilemma persists in nephrology science: developing non-melanoma skin cancer in renal transplant population. J Nephropharmacol 2013; 2(2): 45-46.
16. Baradaran A, Mardani S, Tamadon MR, Shahbazian MR, Beladi Mousavi SS, Amiri M, Ardalan MR, Nasri H. A concurrent primary hypoparathyroidism with bladder carcinoma in a 52-year- old man, who initially presented with macroscopic hematuria. J Parathyr Dis 2015; 3(1): 6-7.
17. Ahmadi A, Noroozi M, Pourhoseingholi MA, Hashemi-Nazari SS. Effect of metabolic syndrome and its components on survival in colorectal cancer: a prospective study. J Renal Inj Prev. 2015; 4(1): 19-23.
18. Arabsalmani M, Mohammadian-Hafshejani A, Ghoncheh M, Hadadian F, Towhidi F, Vafaee K, et al. Incidence and mortality of kidney cancers, and human development index in Asia; a matter of concern. J Nephropathol. 2017;6(1):30-42.
19. Lebrun JJ. The dual role of TGF in human cancer: from tumor suppression to cancer metastasis. ISRN molecular biology. 2012 Dec 24;2012.
20. Markowitz SD1, Roberts AB. Tumor suppressor activity of the TGF-beta pathway in human cancers Cytokine Growth Factor Rev. 1996 Jun;7(1):93-102.
21. Tang B , Böttinger EP, Jakowlew SB, Bagnall KM, Mariano J, Anver MR, Letterio JJ, Wakefield LM. Transforming growth factor-beta1 is a new form of tumor suppressor with true haploid insufficiency. Nat Med. 1998 Jul;4(7):802-7.
22. Blank U, Karlsson S. TGF-β signaling in the control of hematopoietic stem cells. Blood. 2015;125(23):3542-50
23. Abdian N, Allahbakhshian-Farsani M, Khosravi-Farsani S, Ghasemi-Dehkordi P, Kazemi-Sheykhshabani S, Ganji-Arjenaki M, Hashemzadeh-Chaleshtori M. Generation of HSC-Like Cells from Human Embryonic Stem Cells by Inhibition of TGF-βR2 Signaling. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences. 2015 Dec 1;85(4):1017-26.
24. Bagheri L, Hami M, Mojahedi MJ, Ghorban Sabbagh M, Ayatollahi H. Association of metabolic syndrome with serum fibroblast growth factor 21 in kidney transplanted patients. J Renal Inj Prev. 2016;5(2):79-84.
25. Alimohammadi N, Javadian P, Malekpour A, Tahmasebian S. Association of serum fibroblast growth factor 23 with calcium metabolism in patients with end-stage renal disease undergoing hemodialysis. J Nephropathol. 2017;6(4):352-355.
26. Yaghoubi F, Ahmadi F, Lesanpezeshki M, Mahdavi Mazde M. A study on the association of serum fibroblast growth factor-23 with various indices of chronic kidney disease patients not yet on dialysis. J Renal Inj Prev. 2016;5(2):104-107.
27. Baradaran A. Fibroblast growth factor 23 and cardiovascular disease in chronic kidney disease; new trends. J Parathyr Dis. 2018;6(1):1-2.
28. Zhang Y, Alexander PB, Wang XF. TGF-β family signaling in the control of cell proliferation and survival. Cold Spring Harbor perspectives in biology. 2017 Apr 1;9(4):a022145.

29. Sonia B. Jakowlew. Transforming growth factor-β in cancer and metastasis. Cancer Metastasis Rev (2006) 25:435–457. DOI: 10.1007/s10555-006-9006-2.
30. Tang B, Yoo N, Vu M, Mamura M, Nam JS, Ooshima A, Du Z, Desprez PY, Anver MR, Michalowska AM, Shih J. Transforming growth factor-β can suppress tumorigenesis through effects on the putative cancer stem or early progenitor cell and committed progeny in a breast cancer xenograft model. Cancer research. 2007 Sep 15;67(18):8643-52.
31. Cook G, Campbell J, Carr CE, Boyd KS, Franklin IM. Transforming growth factor beta from multiple myeloma cells inhibits proliferation and IL-2 responsiveness in T lymphocytes. Journal of leukocyte biology. 1999;66(6):981-8.
32. Seoane J, Gomis RR. TGF-β Family Signaling in Tumor Suppression and Cancer Progression. Cold Spring Harbor Perspectives in Biology. 2017 Feb 28:a022277.
33. Wolfraim LA, Fernandez TM, Mamura M, Fuller WL, Kumar R, Cole DE, et al. Loss of Smad3 in acute T-cell lymphoblastic leukemia. New England Journal of Medicine. 2004;351(6):552-9
34. Mori N, Morishita M, Tsukazaki T, Giam C-Z, Kumatori A, Tanaka Y, et al. Human T-cell leukemia virus type I oncoprotein Tax represses Smad-dependent transforming growth factor β signaling through interaction with CREB-binding protein/p300. Blood. 2001;97(7):2137-44.
35. Kurokawa M, Mitani K, Imai Y, Ogawa S, Yazaki Y, Hirai H. The t (3; 21) fusion product, AML1/Evi-1, interacts with Smad3 and blocks transforming growth factor-β–mediated growth inhibition of myeloid cells. Blood. 1998 Dec 1;92(11):4003-12.
36. Mitani K. Molecular mechanisms of leukemogenesis by AML1/EVI-1. Oncogene. 2004 May 24;23(24):4263-9.
37. Ford AM, Palmi C, Bueno C, Hong D, Cardus P, Knight D, et al. The TEL-AML1 leukemia fusion gene dysregulates the TGF-β pathway in early B lineage progenitor cells. The Journal of clinical investigation. 2009;119(4):826.
38. Matsuzaki K. Cell Type-specific and Context-dependent TGF-β Signaling: Dialogues between Clinic and Bench. Clin Exp Pharmacol. 2014;4(152):2161-1459.1000152.
39. Nasri H, Dehghan Shahreza F. Defensins usage as novel therapeutic and diagnostic approach. Immunopathol Persa. 2015;1(1):e05.
40. Moghadaszadeh-Ardebili S. The anticancer mechanism of capsaicin on various cancer cell lines. Ann Res Antioxid. 2016;1(1):e06.
41. Shi Y, Massagué J. Mechanisms of TGF-β signaling from cell membrane to the nucleus. Cell. 2003;113(6):685-700.
42. Guo X, Wang X-F. Signaling cross-talk between TGF-β/BMP and other pathways. Cell research. 2009;19(1):71-88.
43. Seoane J. Escaping from the TGFβ anti-proliferative control. Carcinogenesis. 2006;27(11):2148-56.
44. Dong M, Blobe GC. Role of transforming growth factor-β in hematologic malignancies. Blood. 2006;107(12):4589-96.
45. Schwab C1, Harrison CJ. Acute lymphoblastic leukaemia. Methods Mol Biol. 2011;730:99-117. doi: 10.1007/978-1-61779-074-4_8.
46. Macleod K. Tumor suppressor genes. Current opinion in genetics & development. 2000;10(1):81-93.
47. Fortunel NO, Hatzfeld A, Hatzfeld JA. Transforming growth factor-β: pleiotropic role in the regulation of hematopoiesis. Blood. 2000;96(6):2022-36.
48. Mei Dong and Gerard C. Blobe. Role of transforming growth factor- in hematologic malignancies. Blood, 15 June 2006 Volume 107, Number 12. 4589-96.
49. Katz LH, Li Y, Chen J-S, Munoz NM, Majumdar A, Chen J, et al. Targeting TGF-β signaling in cancer. Expert opinion on therapeutic targets. 2013;17(7):743-60.
50. Zhang H-J, Wang H-Y, Zhang H-T, Su J-M, Zhu J, Wang H-B, et al. Transforming growth factor-β1 promotes lung adenocarcinoma invasion and metastasis by epithelial-to-mesenchymal transition. Molecular and cellular biochemistry. 2011;355(1-2):309-14.
51. Renold J. Capocasal, Roberta J. Lamb, Eric C. Vonderheidt, Floyd E. Fox, Alain H. Rook, Peter C. Nowell, and Jonni S. Moore. Reduced surface Expression Of Transforming Growth Factor Receptor Type II In Mitogen-Activated T Cells From Sezary Patients (Sezary Syndrome/Cutaneous T-Cell Lymphoma/Endocytosis/Flow Cytometry). Proc. Natl. Acad. Sci. USA. Vol. 92, pp. 5501-5505, June 1995 Medical Sciences.
52. Biswas S, Chytil A, Washington K, Romero-Gallo J, Gorska AE, Wirth PS, et al. Transforming growth factor β receptor type II inactivation promotes the establishment and progression of colon cancer. Cancer research. 2004;64(14):4687-92.
53. Soulitzis N, Karyotis I, Delakas D, Spandidos DA. Expression analysis of peptide growth factors VEGF, FGF2, TGFB1, EGF and IGF1 in prostate cancer and benign prostatic hyperplasia. International journal of oncology. 2006;29(2):305-14.
54. Dos Reis ST, Pontes-Júnior J, Antunes AA, Sousa-Canavez J, Abe DK, da Cruz JAS, et al. Tgf-β1 expression as a biomarker of poor prognosis in prostate cancer. Clinics. 2011;66(7):1143-7.
55. Lin HK, Bergmann S, Pandolfi PP. Cytoplasmic PML function in TGF-β signalling. Nature. 2004 Sep 9;431(7005):205-11.
56. Jakubowiak A, Pouponnot C, Berguido F, Frank R, Mao S, Massagué J, Nimer SD. Inhibition of the transforming growth factor β1 signaling pathway by the AML1/ETO leukemia-associated fusion protein. Journal of Biological Chemistry. 2000 Dec 22;275(51):40282-7.
57. Wolfraim LA, Fernandez TM, Mamura M, Fuller WL, Kumar R, Cole DE, et al. Loss of Smad3 in Acute T-Cell Lymphoblastic Leukemia. N Engl J Med. 2004 Aug 5;351(6):552-9.
58. Wu Y, Su M, Zhang S, Cheng Y, Liao XY, Lin BY, Chen YZ. Abnormal expression of TGF-beta type II receptor isoforms contributes to acute myeloid leukemia. Oncotarget. 2017 Feb 7;8(6):10037.
59. Zhang HJ, Wang HY, Zhang HT, Su JM, Zhu J, Wang HB, Zhou WY, Zhang H, Zhao MC, Zhang L, Chen XF. Transforming growth factor-β1 promotes lung adenocarcinoma invasion and metastasis by epithelial-to-mesenchymal transition. Molecular and cellular biochemistry. 2011 Sep 1;355(1-2):309-14.
60. Mele V, Muraro MG, Calabrese D, Pfaff D, Amatruda N, Amicarella F, et al. Mesenchymal stromal cells induce epithelial-to-mesenchymal transition in human colorectal cancer cells through the expression of surface-bound TGF-β. International journal of cancer. 2014;134(11):2583-94.