Original Article

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Relationship Between SOX17 Gene Expression and Prognosis in Acute Myeloid Leukemia

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Abstract

Background: Acute Myeloid Leukemia (AML) is a group of heterogeneous malignancies caused by defects in differentiation of hematopoietic cells. SRY-box containing gene 17(SOX17) is a transcription factor which plays an important role in several biological processes, including cardiogenesis, angiogenesis, and lymphopoiesis. Aberrant expression of SOX17 has been detected in solid tumors. This study was performed to investigate the alternations of SOX17 expression in AML patients.

Materials and methods: This case-control study included 54 AML patients who were referred to Molecular Pathology Cancer Research Center of Ghaem Hospital in North East of Iran from October 2011 to May 2016. Patients were classified according to French-American-British (FAB) and World Health Organization (WHO) criteria. RNA was extracted from peripheral blood. SOX17 gene expression was evaluated by real-time quantitative polymerase chain reaction (RQ-PCR).

Results: Over expression of SOX17 was observed in 34 (62.96%) AML patients. No relation was noticed between SOX17 expression and patient survival (p=0.493). In addition, no correlation among patient survival, Sex(p=0.322),hemoglobin(p=0.866) and white blood cell (WBC) (p=0.103).

Conclusion: Based on these results, SOX17 did not have any important role in AML pathogenesis. Thus, it can't be used as a diagnostic and prognostic factor. However, more studies are required to fully elucidate the role of SOX17 in AML.

Keywords: Acute myeloid leukemia, SOX17, Survival

Introduction

Acute Myeloid Leukemia (AML) is extremely heterogeneous group of clonal disorders in hematopoietic precursors. it is characterized by cessation of differentiation and overgrowth of myeloid blasts (1-3).This malignancy is the most common form of acute leukemia in adults(4) and its occurrence increases with age (5). "Wnt/β catenin" is an important pathway for differentiation of fetal hematopoietic and non-hematopoietic stem cells (6-8). Downstream signaling pathway activates different proteins that cause biologic changes within the cells. GATA6, GATA4, FOXA2 and SRY-box containing gene 17 (SOX17) are among those proteins that are activated by "Wnt/β catenin" signaling. GATA6 and SOX17 are two specially necessary proteins for cell differentiation(9). SOX17 has a dual function. It not only has a role in differentiation of hematopoietic stem cells but also works as an antagonist of "Wnt/β catenin" signaling pathway. It causes cell differentiation inhibition and reduces cell division(10). SOX17 protein is a member of "SOX" family. This family is composed of twenty members that are divided into four groups (6, 11-13). Expression of SOX17 in solid tumors is accompanied by

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reduction of cell division, tumor size and metastasis (12-14). On the other hand, SOX17 is known as the main transcription factor that causes fetal hematopoietic cells transformation to adult hematopoietic cells (15). Therefore, it is hypothesized that over expression of SOX17 in adults may lead to leukemia (16) . Previous studies showed that reduction of SOX17 expression in AML patients is associated with poor prognosis(17). Chromosomal translocations induce altered expression of transcription factors which are associated with different prognosis. At the same time, the prognosis is unknown in a large number of cases (18, 19). SOX17 expression was evaluated in AML patients and its relation with hospitalized patient survival in North eastern Iran to find out whether it could be used as a biological marker for AML prognosis.

Materials and Methods

This case-control study included 54 AML patients who were referred to Molecular Pathology Cancer Research Center of Ghaem Hospital in North East of Iran from October 2011 to May 2016. Patients were classified according to French-American-British (FAB) and World Health Organization (WHO) criteria. Disease was diagnosed by two expert haemato-oncologists and the patients were referred to the hematology laboratory of Ghaem Hospital, Mashhad University of Medical Sciences for confirmatory tests. Fifty and four age and sex matched healthy controls were recruited, and their information were collected and used in statistical analysis. Bone marrow and peripheral blood samples were collected in EDTA containing tubes. Consent forms were signed by all patients and healthy controls. All the protocol steps were approved by the ethics committee of MUMS. Information, including age, sex, and laboratory findings was obtained from patients medical records.

Cytogenetical analysis

Patients were divided into two groups cytogenetically: normal and abnormal. Cytogenetic analysis of patients was performed by R banding metaphase method.

RNA extraction and cDNA synthesis

Bone marrow mononuclear cells or peripheral blood cells of AML patients were isolated by gradient ficole method. Total RNA was extracted using RNA extraction Kit (Tri-pure Kit, Roch Company) and stored at -70 ℃. cDNA synthesis was performed according to Kit's guidelines (RNX-Plus solution kit number RN7713C).

Materials required for the RT-PCR included 2 µgs RNA, 4 µgs reaction buffer, 5 M (X), one µg RiboLock RNase inhibitor, 2 µgs dNTP, one µg Reverse Transcriptase, one µg forward primer, one µg reverse primer and 9 µgs distilled water (DEPCE-water). The RT-PCR conditions included 10 minutes at 25 ° C, 60 min at 42 ° C and finally, at a tempreture of 70 ℃ the reaction stopped. Nanodrop 2000 was used to check the quality and quantity of synthesized cDNA.

Real time-PCR

SYBR Green PCR method (SYBR Green real-time PCR) was used to investigate the expression of SOX17. SOX17 gene expression was measured using $2^{-\Delta\Delta CT}$ method and GAPDH reference gene. Primers used for SOX17 gene were as follows: forward, 5′-TGCTGGGCAAGTCGTGGA-3′ and reverse, 5′-CCACTACCGCGACTGCCAGA-3′. Furthermore, the forward and reverse primer sequences for the GAPDH gene were TGCACCACCAACTGCTTAGC and GCATGGACTGTGGTCATGAG, respectively.

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Real time-PCR reaction was performed at a volume of 20 ul. Materials needed for this reaction included 10 ul CYBER Green Katara , 0.5 µl 10 Pico molar forward primer, 0.5 µl 10 Pico molar reverse primer, 2 µl cDNA, and 7 µl double-distilled water. Initial denaturing step was performed at 95° C for 30 seconds, followed by 40 cyclesat64° C for 30 seconds and finally 40 cycles of extension step at 72° C for 30 seconds. Melting curve analysis was performed to ensure the specificity of reaction steps.

Statistical analysis

Statistical analysis was performed by SPSS (version 16). Student T-test and Mann-witheny test were used to assess significance of differences between SOX17 gene expression and internal control gene (GAPDH). Kaplan-Meier and Cox regression tests were applied to examine the relationships between patient survival and gene expression. *P* values <0.05 were considered significant.

Results

This cross-sectional case-control study included 54 AML patients. Concerning sex, 20 (37 %) of the patients were female and 34 (63%) were male. Patients' mean age was 36.24±23.46. The minimum age was six months and the maximum was 74 years. The mean white blood cell (WBC) and platelet (Plt) counts and Hb concentration were 48.74±53.52, 65.57±53.59, and 7.75±2.32; respectively.

In this study, SOX17 gene expression was examined in AML patients and healthy controls. Patients were divided into two groups based on SOX17 expression: a group with increased expression, and the other with decreased expression of SOX17. No significant correlations were found between SOX17 expression and age, gender, WBC and Plt counts in both AML groups. However, a significant correlation was observed between Hemoglobin (Hb) concentration and SOX17 gene expression in the AML group ($p<0.039$) (Table I). Twenty four-month survival of AML patients was calculated (range between 2-48 months). Survival mean was 31.30 ± 1.95 in females and 33.17 ± 2.42 in males. No significant correlation was found between SOX17 expression and patient survival. Moreover, the overall survival was examinedin cohort with normal karyotypes and no correlation was observed between SOX17 expression and patient survival (Figure 1). No correlation was found among Sex, hemoglobin, and WBC with survival (Table II).

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Table I: Correlation between SOX17expression and patients' parameters.

Patients' parameters				
SOX17 expression	Low	high	**Total**	**p-Value**
Sex, male/female	10/10	21/13	31/23	.569
Median age, years	35.33	31.97	36.24±23.46	.594
Median WBC, $\times 10^9$/L (range)	61.34	41.33	48.74	.213
Median platelets, $\times 10^9$/L (range)	63.40	66.85	65.67	.807
HG	6. 96	8.22	7.75	.039
FAB				.526
M0	0	1	1	
M1	6	8	14	
M2	3	8	11	
M3	3	5	8	
M4	4	4	8	
M5	5	4	9	
M6	0	2	2	
M7	1	0	1	
WHO				.147
AML with t(8;21)	1	3	4	
AML with t(15;17)	1	7	8	
AML with t(6;9)	0	2	2	
AML with inv(16)	2	1	3	
AMLwith normal cytogenetic	15	22	37	.269
*P<0.05				

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Factor	P value
SOX17 expression	.493
Hb	.866
Sex	.322
WBC	.103
***P<0.05**	

Table II: correlation between Hb, Sex and WBC with overall survival in AML patients

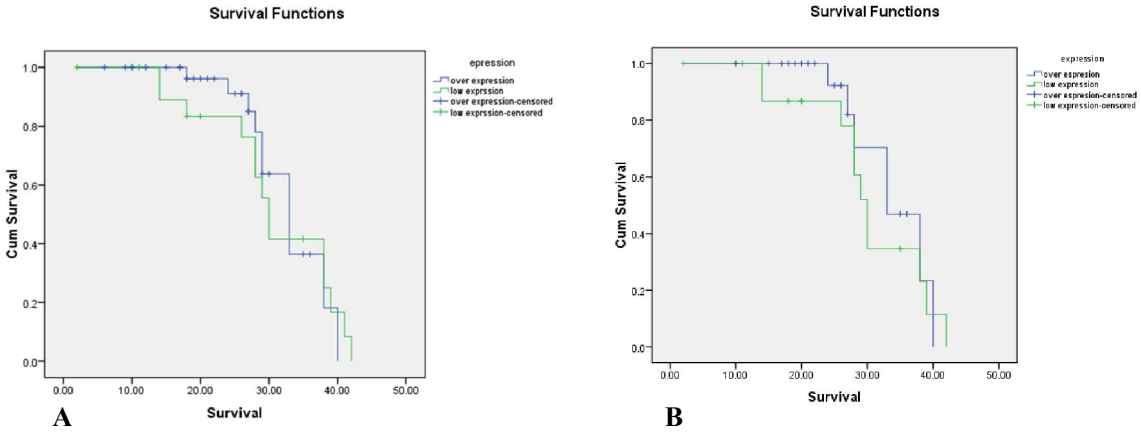

Figure 1. Kaplan-Meier survival curve on the two groups based on SOX17 expression. (A) All patients; (B) cytogenetically normal patients.

Discussion

Variations of molecular genetics are not only a basis to predict prognosis, but also a potential molecular target for leukemia treatment. Malignancy transformation process is complicated in leukemia(20). Some important prognostic cytogenetic and molecular markers, including repetitive translocations such as t(15;17), internal tandem duplication of flt3 gene, NPM1 gene mutations, partial tandem duplication of MLL gene, mutation in CCAAT/enhancer binding protein alpha(CEBPA), and Wilms tumor 1(WT1) genes are discovered (21).

Gene expression alterations have been observed in solid tumors such as colon (12, 22) and breast cancers(23, 24). Decreased expression of SOX17 is associated with tumor progression and poor prognosis in solid tumors. Ying Kuo et al., showed low expression of SOX 17 in esophageal cancer. They showed a link between SOX 17 expression and tumor progression(25). According to the role of SOX17 gene in fetal Hematopoiesis, it is thought that increased expression of SOX17 is associated with increased risk of AML(16). However, later studies showed that hyper-methylation of SOX 17 gene promoter is associated with poor prognosis in blood malignancies. Fan R et al., detected SOX17 gene methylation in MDS patients. In this study, 164 patients with MDS were examined by methylation-specific PCR method. SOX17 gene was

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methylated in 96 patients. According to WHO criteria and IPSS, SOX17 gene was highly methylated in patients who had poor prognostic indicator (17).

A similar study was done by Ghasemi et al., in 2015 (26). They identified SOX17 gene promoter hyper methylation in AML patients. They suggested that gene hyper-methylation is associated with decreased SOX 17 expression and poor prognosis. They did not find any relation between SOX17 methylation and recurrence occurrence. They demonstrated that gene methylation is significantly higher in AML-M1 group. In this study, however, no correlation was discovered between gene expression and various subgroups of AML based FAB and WHO classifications. Hemoglobin concentration in cases with increased expression of SOX17 was significantly higher in comparison with the cases with decreased expression of SOX17 (P=0.039). This finding was not consistent with Ghasemi's study. They did not observe any relations between laboratory findings and gene methylation. There is no data regarding patient survival and SOX17 gene expression in hematologic malignancies. Tang et al., examined SOX17 gene expression in patients with AML (17); they concluded that decreased expression of SOX17 gene is associated with decreased survival rate of patients. In comparison with Tang's study, these results indicated that there is no significant correlations between SOX17 gene expression level and patient survival rate (P=0.493). In this research, SOX17 expression was not significantly correlated with age, sex and hematologic parameters; but in contrast with findings of current investigation, Tang's study showed that hemoglobin levels are significantly associated with increased expression of SOX17. Tang showed that average survival rate of patients was 1-19 months. In comparison with their study, a longer period study was designed. Based on the results of this study, there was no significant correlation between patient survival rate and others parameters. The median age of Tang's study was 55 years that is higher than this study (36.24±23.46). Maybe younger patients and longer period study led to this difference between the current investigation and Tang's study.

Conclusion

These differences may be due to lower sample size and shorter survival period in this study. Finally, this study showed that there is no significant correlation between SOX17 gene expression level and survival rate of AML patients. Furthermore, survival study of patients did not show any significant relationships between SOX17 gene expression and survival rate of normal cytogenetic AML patients. It is better to perform further studies with larger sample size and quantitative real-time PCR methods to determine the role of SOX17 gene in patient's prognosis.

Conflict of interest

The authors report no conflict of interest.

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