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HMGB1 polymorphisms in acute lymphoblastic leukemia

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Abstract

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy and the leading cause of childhood death in contrast to the 90% cure rates. ALL includes different subtypes described by interrupt collections of somatic chromosomal alterations and sequence mutations that disrupt normal body functions such as lymphoid maturation, cell-cycle regulation, and tumor suppression. Having a significant role in several cancers, the high mobility group box-1 (HMGB1) gene considered an important gene in the development of tumors. Herein, the genetic role of HMGB1 was studied in the 49 Iranian patients with newly diagnosed ALL using Sanger sequencing of HMGB1 coding regions (exons 2 to 5). The results showed that none of the subjects in the study had any promising variants in the coding sequences of the HMGB1. These findings suggest that HMGB1 is not directly associated with ALL incidence and behavior. Further investigations using a large group of patients with different races and ethnicities are required to analyze the possible role of HMGB1 gene polymorphisms in ALL patients.

Introduction

Acute lymphoblastic leukemia (ALL) is the most common pediatric malignancy and the leading cause of childhood death in contrast to the 90% cure rates [1]. ALL includes different subtypes described by distinct constellations of somatic chromosomal alterations and sequence mutations that disrupt lymphoid maturation, cell-cycle regulation, kinase signaling, tumor suppression, and chromatin modification[2]. Of these genetic changes, some influence the risk of treatment failure and relapse in addition to leading to leukemogenesis. Notably, *KMT2A* (previously known as *MLL*) rearrangement, *BCR-ABL1* mutation, *ETV6-RUNX1* (due to t(12;21)) fusion, and activating kinase alterations in Ph-like ALL are correlated with poor disease outcome. Deletion or mutation of the*IKZF1* lymphoid transcription factor gene also confers a poor prognosis. Another common translocation is t(1;19), leading to*TCF3-PBX1 (E2A-PBX1)* fusion, which occurs in approximately 5% of childhood cases as well as in adult ALL [3-5].

Several single gene studies on ALL have been recently focused on single nucleotide polymorphism. However, the role of high mobility group box-1*(HMGB1)* gene polymorphisms has not been investigated in ALL patients so far. HMGB1 protein is a highly conserved ubiquitous protein which is present in high concentration in the nucleus and cytoplasm of mammalian cells. This protein, previously known as a DNA binding protein, has a crucial role in the nucleosome structure maintenance and gene transcription regulation [6,7].

The aim of the current study was to detect the *HMGB1* gene polymorphisms among the Iranian patients with new-onset ALL, as well as it's relation with the disease behavior, and patients' survival and outcome.

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Materials and Methods

The study was carried out in the Children's Medical Center in Tehran university of medical sciences. In this study, 49 newly diagnosed ALL patients consist of 21 females and 28 males with a median age of 28 years (range: 14 to 80 years) were included. The diagnosis of the patients was made according to the standard morphological examinations and immunophenotyping. Patient recruitment and all experimental protocols used in this study complied with the Declaration of Helsinki and were approved by the Ethics Committee of the Tehran University of Medical Sciences. Written informed consent was obtained from the patients prior to entering the study.

Peripheral whole blood samples were collected from each patient at the time of diagnosis and DNA was isolated by the standard salting-out method. Primers were designed for 4 coding exons (exons 2-5) of the*HMGB1* gene. DNA samples were amplified in a volume of 25 µl, containing 40 ng of DNA template, 10 µM of each reverse and forward primers, and 12 µl of Taq DNA polymerase 2Xmastermix (Ampliqon). The cycling conditions were as follows: initial denaturing, 5 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 60 °C, and 30 s at 72 °C. An additional extension step of 10 min at 72 °C was also performed. Afterward, PCR products were loaded and visualized on a 1% agarose gel using SYBR-safe dye and subsequently proceeded Sanger sequencing using Applied Biosystems 3130 Genetic Analyzer. Ultimately, the sequenced data of each individual were analyzed for searching for any possible variant in the exons.

Results

In the current study, all five exons of the *HMGB1* gene were analyzed in the subjects. Assuming the potential role in different malignancies according to previous studies, *HMGB1* gene polymorphisms were evaluated in newly diagnosed ALL patients. The results revealed that none of the 49 enrolled patients had any variants in the *HMGB1* gene.

Discussion

Acute lymphoblastic leukemia (ALL) is the most prevalent pediatric cancer and the leading cause of childhood death [1]. In this study, the *HMGB1* gene polymorphisms were used to study in 49 Iranian ALL patients with the purpose of finding the role of this gene in the development of ALL. After analyzing of all exons of the *HMGB1* in the subjects, no promising variant was identified in the coding sequences of the HMGB1. These findings suggest that HMGB1 is not a clinically significant gene in ALL and other genetic changes may have a role in these patients.

The *HMGBs* are a highly conserved family including four members (*HMGB1* , *HMGB2* , *HMGB3* , and *HMGB4*). It is known that knocking out the *HMGB1* , *HMGB2* , and *HMGB3*genes in mice results in noticeable phenotypic changes, although the encoded proteins share approximately 80% amino acid sequence identity. Each HMGB has two DNA binding domains termed as HMG boxes A and B. HMGB1–3 include an acidic C-terminal tail, whereas HMGB4 lacks this tail [8]. *HMGB1* , as a member of mammalian HMG-box family, include tandem homologous DNA binding domains, called HMG-box A and B. These domains comprised of about 80 amino acids folded into three α-helices that adopt a characteristic L-shaped structure and followed by a linker abundant in basic amino acid residues and a C-terminal acidic tail contains about 30 consecutive aspartate and glutamate residues[8].

Besides performing nuclear functions, HMGB1 has a significant extracellular function as damage-associated molecular pattern molecules (DAMPs) [9]. Extracellular HMGB1 functions as a DAMP to alert the innate immune system by recruiting mesangioblasts, stem cells and, smooth muscle cells [10].

Primary studies demonstrated the role of HMGB1 as a late mediator of sepsis. Currently, it has been exhibited as a danger signal involved in the pathogenesis of various non-infectious inflammatory diseases[11,12]. It has been also proved that HMGB1 plays pivotal rules in tissue repair, remodeling, and preconditioning that these findings made HMGB1 as an important protein in danger signal[11].

The HMGB1 expression is higher in myeloid cells compared to lymphoid cells. HMGB1 expression is upreg-

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ulated in cancer cells, however, it is downregulated during aging, suggesting a critical role in development and cancer [9]. As a nuclear DNA-binding protein, HMGB1 is involved in the transcription of several cancer-related genes, including E-selectin [13], TNFα [14], insulin receptor [15], and BRCA [16]. In addition, in necrotic cancer cells, HMGB1 releases into tumor microenvironment that lead to chronic inflammatory and reparative responses that consequently leads to cancer cell survival and metastasis[17]. Having a role in metastasis, HMGB1 correlated with poor prognosis in a variety of cancers including breast[18], colon [19], pancreas[20], and prostate [21]. However, the role of HMGB1 has not been studied in ALL so far, plays an important role in tumor development, growth, and metastasis. Despite HMGB1 that no evidence is available for proving its role in ALL development, another member of the HMG family, HMGB3 has shown promising effects in improving ALL. It has been proved that HMGB3 in the fusion with NPU98 (HMGB3-NPU98 fusion protein) is an oncogene found in leukemia and augments malignant transformation in recipient mice [22]. It is known that HMGB1 has been secreted and accumulates in cell membranes during murine erythroleukemia cell differentiation [23]. The N-terminal 18 amino acids of the hydrophilic acylated surface protein B , an unclassical secretory signal peptide, could deliver HMGB1 on the cell surface, efficiently [24]. In erythroblast-macrophage contact, HMGB1 is involved in macrophage-mediated erythroid proliferation and maturation in a homophilic manner [25]. It has been studied that during platelet activation, HMGB1 translocases to the membrane and is then released [26], which mediates neutrophil extracellular traps formation and function [27,28]. Extracellular HMGB1 leads to chronic lymphocytic leukemia differentiation[29]. It is exhibited that retreatment with HMGB1 results in endotoxin and lipoteichoic acid tolerance in bone marrow-derived macrophages and the acute monocytic leukemia cell line THP-1 through NF-KB activity downregulation [9,30]. Interestingly, it is revealed that miR181a impedes the expression of HMGB1 in T- and B ALL cells and consequently results in a decline in cell proliferation and metabolic activity [31]. Moreover, HMGB1-mediated autophagy augments chemoresistance in cancer cells, including leukemia, colon cancer, gastric cancer, ovarian cancer, osteosarcoma, and pancreatic cancer [9]. Despite the comprehensive study of HMGB1 both biologically and clinically, there is not any promising evidence suggesting the role of HMGB1 in ALL. Kang, R. et al showed that the HMGB1 serum levels were significantly higher in ALL initial treatment group compared to the healthy control group and ALL complete remission group. Interestingly, HMGB1 levels had no significant differences between the healthy control group and ALL complete remission group. Moreover, it is shown that HMGB1 treatment of K562 cells, led to secretion and augmentation in the TNF-α level. Using JNK (SP600125), MEK (PD98059), and p38 MAPK (SB203580) inhibitors resulted in HMGB1-induced TNF-α secretion arrest. The serum HMGB1 measurement in the assessment of childhood ALL is beneficial due to HMGB1 stimulates leukemic cells to secrete TNF-alpha through a MAPK-dependent mechanism [32].

In sum, HMGB1 has been studied in several biological and medical conditions majorly cancers such as colon, pancreatic, breast, and prostate cancers. A few studies investigated the role of the HMGB1 gene in leukemia however, lacking conclusive information around the hematological malignancies. Studying a large group of ALL patients and from different ethnicities may noticeably help understanding the HMGB1 role in ALL patients.

Conflict of Interest

The authors declare that they have no conflict of interests.

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Authors' contributions

"Elham Rayzan" contributed to the laboratory works and was in charge of the project management. *"Saeed Farajzadeh Valilou"*drafted the manuscript and contributed to the laboratory works. *"Sara Hemmati"* and *"Amin Sadeghi"* helped in improving the study design and proposal preparation. *"Hamid Farajifard"*designed the primers and analyzed the sanger results. *"Sepideh Shahkarami"* managed the patients' recruitment and samplings, proposal drafting, and sanger results analysis. *"Nima Rezaei"* defined the study protocol and supervised the whole project. All authors read, critically revised, and approved the final manuscript.

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