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Does Endoplasmic Reticulum (ER) Stress Contribute to T-cell Exhaustion in B-ALL?

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ABSTRACT

Background: Glucose deprivation in T lymphocytes can trigger compensatory metabolic pathways, potentially contributing to T-cell exhaustion. Additionally, it may induce the unfolded protein response (UPR), ultimately resulting in endoplasmic reticulum (ER) stress.

Objectives: To examine the transcriptional profiles of endoplasmic reticulum (ER) stress markers and T-cell exhaustion indicators in CD8+ T lymphocytes isolated from B-ALL patients.

Methods: Peripheral blood samples were collected from 22 untreated B-ALL patients and 22 healthy controls. Magnetic Activated Cell Sorting (MACS) was used to isolate CD8+ T lymphocytes. The relative gene expression was then assessed using qRT-PCR with primers specific to XBP1, CHOP, GLUT1, and T-bet.

Results: The ER stress response was significantly activated in CD8+ T lymphocytes from B-ALL patients, as evidenced by significant increase in both XBP1 and CHOP transcript levels, relative to normal donors. Although GLUT1 mRNA expression was significantly higher than in control groups, T-bet expression showed no significant difference between the two groups.

Conclusion: Collectively, our gene expression data suggest ER stress activation in CD8+ T lymphocytes from B-ALL patients. These findings warrant further investigation into ER stress-related signaling pathways and their potential role in promoting T-cell exhaustion in B-ALL.

Keywords: Acute Lymphoblastic Leukemia, CHOP, ER Stress, T-cell Exhaustion, XBP1

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INTRODUCTION

B-cell acute lymphoblastic leukemia (B-ALL) is a heterogeneous and life-threatening disease primarily detected in children and juveniles. This subtype of leukemia features malignant overgrowth of early-stage B-cell progenitors in the hematopoietic niche (1). Recent advances in molecular characterization, risk-stratified treatment protocols, and innovative therapies have significantly improved survival rates in B-ALL, particularly in pediatric cases (2). In addition to hematopoietic stem cell transplantation, radiation therapy and chemotherapy (3), increasing research have focused on immunotherapeutic approaches (4, 5). In certain pathological conditions, such as acute infections and cancers, persistent antigen exposure can induce T-cell exhaustion. Exhausted T lymphocytes overexpress immune-checkpoint receptors (ICRs) that bind their ligands. Ultimately, the proliferative capacity, cytokine production, and cytotoxic function of T lymphocytes are impaired (6).

Growing evidence suggest that metabolic dysregulation, particularly those resulting from glucose deprivation in T lymphocytes, plays a pivotal role in driving T-cell exhaustion. In this regard, exhausted T lymphocytes show decreased levels of glycolysis and GLUT1 expression, mediated through suppression of mTOR signaling pathways (7, 8). Within these cells, reduced GLUT1 expression results in decreased glucose uptake, impairing both glycolysis and oxidative phosphorylation (OXPHOS). Consequently, exhausted T lymphocytes preferentially switch metabolic pathways from glycolysis and OXPHOS to fatty acid oxidation (FAO) (7-9). Following the synthesis of new proteins by ribosomes and transportation into the endoplasmic reticulum (ER) lumen, they undergo a process called protein folding. N-linked glycosylation is a form of post-translational modification associated with protein folding. Glucose is crucial for N-glycosylation in the modulation of folding new protein (10). Glucose deprivation compromises the protein folding, leading to accumulation of misfolded proteins in the ER lumen that stimulates the activation of ER stress sensors, such as protein kinase RNA-like ER kinase (PERK), inositol-requiring enzyme 1 alpha (IRE-1a) and activating transcription factor 6 (ATF6) (11). Exhausted T lymphocytes experience reduced glucose uptake resulting from downregulated GLUT1 expression (7, 8). Therefore, these findings demonstrate disruption in protein folding and consequent ER stress (12, 13). ER stress induces the unfolded protein response (UPR), which is a compensatory signaling network that aims to resolve folding stress and recover ER homeostasis, supporting cell survival and adaptation (14). UPR activates various transcription factors to modify the gene expression of multiple proteins. X-box binding protein (XBP1) acts as a crucial effector molecule in the cellular stress response pathway, becoming functionally active through IRE-1α-mediated signaling during endoplasmic reticulum stress. XBP1 binds to both the pdcd1 and CD244a genes' promoters and enhances the expression of PD-1 and CD244 genes (15). Additionally, the C/EBP homologous protein (CHOP) is activated through dual ER stress transduction mechanisms: the PERK- eIF2α-ATF4 signaling axis and the ATF6 proteolytic activation pathway. CHOP downregulates TBX21 gene expression directly and diminishes T-bet protein expression. T-bet is the main transcription factor of activated T-cells (16).

Little is known about the association between GLUT1, ER stress genes, and ICRs in exhausted T lymphocytes in B-ALL. This study aimed to assess the mRNA expression levels of GLUT1, XBP1, CHOP, and T-bet in CD8+ T lymphocytes from individuals with B-ALL. The findings of this study could help identify new targets for immunotherapy.

MATERIALS AND METHODS

Patients and Controls

In line with previous studies (17-19), this research included 22 individuals diagnosed

with B-cell acute lymphoblastic leukemia (B-ALL) from the Hematology and Oncology departments of Imam Khomeini Hospital and Bou-Ali Sina Hospital, both affiliated with Mazandaran University of Medical Sciences in Iran. Additionally, 22 healthy volunteers were included for comparison, as a control group. Patient diagnoses were confirmed based on standardized World Health Organization (WHO) guidelines, which involved evaluations of blood cell counts, cellular morphology, and immunophenotyping analyses conducted on either peripheral blood or bone marrow specimens (20). The study excluded participants who had persistent viral infections, inherited or acquired immune system disorders, previous diagnoses of other malignancies, or autoimmune conditions. In compliance with Mazandaran University of Medical Sciences' ethical regulations, written informed consent was obtained from all subjects before their participation (Ethics Code: MAZUMS.REC.1402.18135). Blood samples (8–10 mL**)** were collected from each individual using heparin as an anticoagulant to prevent coagulation.

Isolation of CD8+ T lymphocytes by magnetic-activated cell sorting (MACS)

Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples through Ficoll-Histopaque density gradient centrifugation (Biosera, France). After viability assessment with trypan blue staining, CD8+ T lymphocytes were purified from PBMCs using CD8 microbead-based MACS (Miltenyi Biotec, Germany). The purity of the isolated CD8+ T lymphocytes was confirmed through dual-color flow cytometry using anti-CD3-PE and anti-CD8-FITC antibodies (BioLegend, USA) on a Partec PAS flow cytometer (Partec GmbH, Münster, Germany). The isolated CD8+ T-cell populations consistently exhibited >99% purity (21).

Quantitative Reverse-Transcription PCR (qRT-PCR)

Total RNA was isolated from CD8+ T lymphocytes using the FavorPrep Blood/ Cultured Cell Total RNA Mini Kit (Favorgen, Taiwan) following the manufacturer's instructions. RNA concentration and purity were assessed using a nano-spectrophotometer, while integrity was verified by electrophoresis. Subsequently, complementary DNA (cDNA) was generated from the extracted RNA using the cDNA Synthesis Kit (Yekta Tajhiz Azma, Tehran, Iran) according to the manufacturer's protocol. mRNA expression levels of GLUT1, XBP1, CHOP, T-bet, and the reference gene HPRT were quantified using a High Rox real-time PCR master mix (Ampliqon, Denmark) on an ABI StepOne Plus system (Thermo Scientific). Gene-specific primers were designed, and their amplification efficiencies were validated by conventional PCR and further confirmed using LinregPCR software. After normalization to HPRT, the relative expression of XBP1, CHOP, GLUT1, and T-bet was calculated using the Pfaffl method (Table 1) (22).

Table 1. Specific forward and reverse primer pairs for the amplification of genes

Gene Name	Primer Sequence	PCR Product Size
XBP1 (XBP1)	F: AGCAAGTGGTAGATTTAGAAGAA R: TCCTGGTTCTCAACTACAAG	97
TBX21 (T-bet)	F: CCAGATGATTGTGCTCCA R: CTGAGTAATCTCGGCATTCT	172
DDIT3 (CHOP)	F: CTGGAAATGAAGAGGAAGAATC R: CTGACTGGAATCTGGAGAG	137
SLC2A1 (GLUT1)	F: GAAGAGAGTCGGCAGATG R: AGTAATAGAAGACAGCGTTGAT	145
HPRT1 (HPRT)	F: GGACTAATTATGGACAGGACTG R: GCTCTTCAGTCTGATAAAATCTAC	195

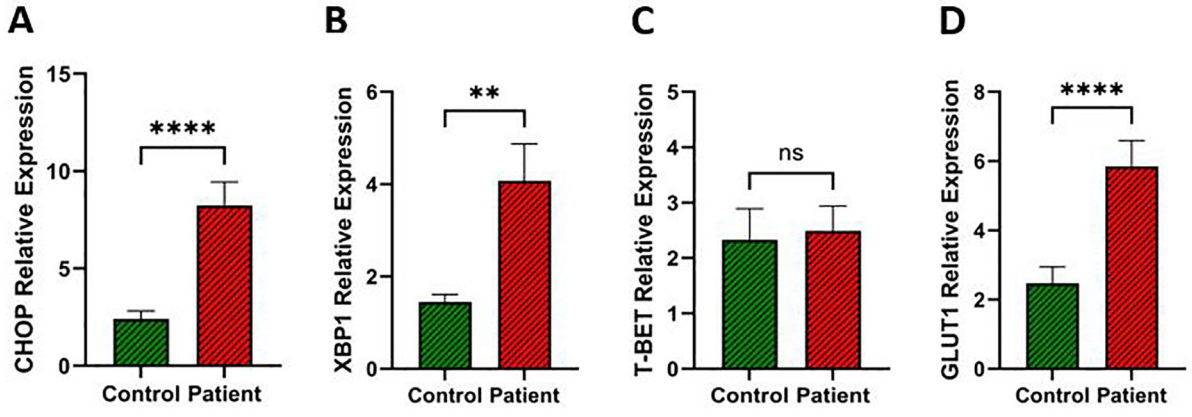

Fig. 1. Relative mRNA expression of CHOP, XBP1, T-bet, and GLUT1 in B-ALL patients and healthy donors. The transcript levels of CHOP, XBP1, T-bet, and GLUT1 were measured using real-time reverse transcription PCR (qRT-PCR). All expression data were normalized to the reference gene HPRT and are presented as relative expression values. Quantitative data is expressed as mean±SEM. ns: non-significant. *P<0.05 ؛**P<0.01 ؛*** P<0.001؛ ****P<0.0001. CHOP: C/EBP homologous protein; XBP1: X-box binding protein-1; GLUT1: Glucose transporter type 1

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 6 software (San Diego, CA, USA). Values represent means±SEM. Since the data were not normally distributed, intergroup differences were calculated using the Mann-Whitney U test.

RESULTS

CD8+ T Lymphocytes Experience ER Stress

We first looked at the changes in mRNA expression of CHOP and XBP1, two important regulators of ER stress, CD8+ T lymphocytes taken from patients. As illustrated in Fig. 1A and B, our findings revealed that the levels of CHOP and XBP1 transcript in CD8+ T lymphocytes from B-ALL patients were significantly higher compared to the control group, suggesting that these cells are undergoing ER stress.

T-bet and GLUT1 mRNA Expression in Patients and Healthy Donors

We then examined the mRNA expression of T-bet and GLUT1, two important factors in ER stress, in CD8+ T lymphocytes derived from patients with B-ALL. Although the results showed similar T-bet mRNA expression patterns in both B-ALL patients and control subjects, a significant increase in GLUT1 mRNA expression was observed in patient-derived CD8+ T lymphocytes. (Fig. 1C and D).

DISCUSSION

This study examined the potential induction of ER stress in T lymphocytes derived from B-ALL patients, focusing on the expression levels of key ER stress-related genes. In T lymphocytes, there are numerous reasons that can lead to ER stress, such as glucose deprivation. Cancer cells consume the most glucose units to survive and maintain their escape mechanisms. One of these mechanisms is T-cell exhaustion which might be connected to ER stress. In some cancers, it has been shown that the XBP1 and CHOP genes are upregulated and activated due to ER stress, leading to impaired glucose uptake, metabolic alterations, and reduced T-cell infiltration into the tumor microenvironment (16, 23). Similarly, our results demonstrate that the transcript levels of CHOP and XBP1, two critical downstream sensors of ER stress, were significantly elevated in patient-derived CD8+ T cells compared to those from healthy individuals. These findings support our hypothesis that ER stress may be prominently induced in CD8+ T lymphocytes in B-ALL.

Furthermore, GLUT1 is an essential protein involved in regulating ER stress and contributing to T-cell exhaustion. One of the mechanisms that could lead to ER stress is glucose deprivation, which is expected to reduce both GLUT1 expression and activity. Consequently, T lymphocytes experience lower glycolysis, ultimately leading to ER stress. In this regard, Guerrero et al. showed that overexpression of GLUT1 in CAR-T-cells could alleviate T-cell exhaustion (24). However, Schurich et al. reported that in HBV infection, a chronic viral disease, exhausted T lymphocytes upregulate GLUT1, which is consistent with PD-1 expression (25). In our study, we did not observe a significant difference in T-bet transcript levels in CD8+ T cells when comparing B-ALL patients with healthy controls. We have previously demonstrated that CD8+ T lymphocytes exhibit heterogeneity and function as progenitor exhausted T lymphocytes in B-ALL (manuscript under review). We have also indicated that the pattern of ICR expression in CD8+ T lymphocytes differs in B-ALL compared to solid tumors (21). Therefore, it is suggested that in B-ALL, unlike in some solid tumors, T lymphocytes are not fully exhausted; however, further investigations are needed.

In conclusion, our findings indicate that ER stress occurs in B-ALL, as CHOP and XBP1, two key ER stress markers, are significantly elevated in patient-derived CD8+ T cells compared to those from healthy individuals. Additional research is necessary to assess the effects of ER stress on T-cell exhaustion. A proper understanding of the mechanistic link between ER stress and T-cell exhaustion in B-ALL could help identify new therapeutic strategies for this disease.

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AUTHORS' CONTRIBUTION

Amir Kahrizi: conceptualization, data management, formal analysis, research, methodology, pro-ject management, resources, software, Writing: original draft; writing: editing and review. Armin Akbar: conceptualization, research, methodology, resources. Ahmad Najafi: conceptualization, research, methodology. Hossein Asgarian-Omran: methodology, project management, resources. Hossein Karami: resources; Mohammad NaderiSorki: resources; Alireza Karimi: conceptualization; Mohsen Tehrani: conceptualization, data management, formal analysis, research, methodology, project management, resources, software, supervision, validation, and visualization; writing: edit-ing and review.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Huang FL, Liao EC, Li CL, Yen CY, Yu SJ. Pathogenesis of pediatric B-cell acute lymphoblastic leukemia: Molecular pathways and disease treatments. Oncol Lett. 2020;20(1):448-54.
2. Mathews V, Korula A, Chakrapani A, Bhurani D, Bhattacharyya J, Sengar M, et al. Management of B-cell lineage acute lymphoblastic leukemia: expert opinion from an Indian panel via Delphi consensus method. Front Oncol. 2023;13:1171568.
3. García-Escobar I, Sepúlveda J, Castellano D, Cortés-Funes H. Therapeutic management of chronic lymphocytic leukaemia: state of the art and future perspectives. Crit Rev Oncol Hematol. 2011;80(1):100-13.
4. Jasinski S, De Los Reyes FA, Yametti GC, Pierro J, Raetz E, Carroll WL. Immunotherapy in Pediatric B-Cell Acute Lymphoblastic Leukemia: Advances and Ongoing Challenges. Paediatr

Drugs. 2020;22(5):485-99.
5. Aureli A, Marziani B, Venditti A, Sconocchia T, Sconocchia G. Acute Lymphoblastic Leukemia Immunotherapy Treatment: Now, Next, and Beyond. Cancers. 2023;15(13):3346.
6. Yi JS, Cox MA, Zajac AJ. T-cell exhaustion: characteristics, causes and conversion. Immunology. 2010;129(4):474-81.
7. Dabi YT, Andualem H, Degechisa ST, Gizaw ST. Targeting Metabolic Reprogramming of T-Cells for Enhanced Anti-Tumor Response. Biologics. 2022;16:35-45.
8. Zheng K, Zheng X, Yang W. The Role of Metabolic Dysfunction in T-Cell Exhaustion During Chronic Viral Infection. Front Immunol. 2022;13:843242.
9. Franco F, Jaccard A, Romero P, Yu Y-R, Ho P-C. Metabolic and epigenetic regulation of T-cell exhaustion. Nature Metabolism. 2020;2(10):1001-12.
10. Braakman I, Hebert DN. Protein folding in the endoplasmic reticulum. Cold Spring Harb Perspect Biol. 2013;5(5):a013201.
11. Malhotra JD, Kaufman RJ. The endoplasmic reticulum and the unfolded protein response. Semin Cell Dev Biol. 2007;18(6):716-31.
12. Hurst KE, Lawrence KA, Essman MT, Walton ZJ, Leddy LR, Thaxton JE. Endoplasmic Reticulum Stress Contributes to Mitochondrial Exhaustion of CD8(+) T Cells. Cancer Immunol Res. 2019;7(3):476-86.
13. Li A, Song NJ, Riesenberg BP, Li Z. The Emerging Roles of Endoplasmic Reticulum Stress in Balancing Immunity and Tolerance in Health and Diseases: Mechanisms and Opportunities. Front Immunol. 2019;10:3154.
14. Oslowski CM, Urano F. Measuring ER stress and the unfolded protein response using mammalian tissue culture system. Methods Enzymol. 2011;490:71-92.
15. Ma X, Bi E, Lu Y, Su P, Huang C, Liu L, et al. Cholesterol Induces CD8(+) T Cell Exhaustion in the Tumor Microenvironment. Cell Metab. 2019;30(1):143-56.e5.
16. Cao Y, Trillo-Tinoco J, Sierra RA, Anadon C, Dai W, Mohamed E, et al. ER stress-induced mediator C/EBP homologous protein thwarts effector T cell activity in tumors through T-bet repression. Nat Commun. 2019;10(1):1280.
17. Chauvin JM, Pagliano O, Fourcade J, Sun Z, Wang H, Sander C, et al. TIGIT and PD-1 impair tumor antigen-specific $CD8^+$ T cells in melanoma patients. J Clin Invest. 2015;125(5):2046-58.
18. Gros A, Robbins PF, Yao X, Li YF, Turcotte S, Tran E, et al. PD-1 identifies the patient-specific $CD8^+$ tumor-reactive repertoire infiltrating human tumors. J Clin Invest. 2014;124(5):2246-59.
19. Toor SM, Murshed K, Al-Dhaheri M, Khawar M, Abu Nada M, Elkord E. Immune Checkpoints in Circulating and Tumor-Infiltrating CD4(+) T Cell Subsets in Colorectal Cancer Patients. Front Immunol. 2019;10:2936.
20. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375-90.
21. Akbar A, Asgarian-Omran H, Valadan R, Dindarloo MM, Najafi A, Kahrizi A, et al. Expression of Galectin-9-related immune checkpoint receptors in B-cell acute lymphoblastic leukemia. Iran J Basic Med Sci. 2023;26(12):1468-74.
22. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001;29(9):e45.
23. Song M, Sandoval TA, Chae CS, Chopra S, Tan C, Rutkowski MR, et al. IRE1α-XBP1 controls T cell function in ovarian cancer by regulating mitochondrial activity. Nature. 2018;562(7727):423-8.
24. Guerrero JA, Klysz DD, Chen Y, Malipatlolla M, Lone J, Fowler C, et al. GLUT1 overexpression in CAR-T cells induces metabolic reprogramming and enhances potency. Nat Commun. 2024;15(1):8658.
25. Schurich A, Pallett LJ, Jajbhay D, Wijngaarden J, Otano I, Gill US, et al. Distinct Metabolic Requirements of Exhausted and Functional Virus-Specific CD8 T Cells in the Same Host. Cell Rep. 2016;16(5):1243-52.