TURKISH JOURNAL of ONCOLOGY

Turk J Oncol 2023;38(4):488–95
doi: 10.5505/tjo.2023.3968

ORIGINAL ARTICLE

A Potential Target for Treating Acute Myeloid Leukemia: XBP1 involved in Endoplasmic Reticulum Stress

Nezahat Ece BİBEROĞLU,[1] Şeyma PUNAR,[1] Sema Sırma EKMEKÇİ,[1] Muhlis Cem AR,[2] Ayşegül ÜNÜVAR,[3] Zeliha EMRENCE,[1] Tuğrul ELVERDİ,[2] Ayşe SALİHOĞLU,[2] Zeynep KARAKAŞ,[3] Neslihan ABACI[1]

[1]Department of Genetics, İstanbul University, Aziz Sancar Institute of Experimental Medicine, İstanbul-*Türkiye*
[2]Department of Internal Medicine, İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, İstanbul-*Türkiye*
[3]Department of Pediatric Hematology and Oncology, Istanbul Faculty of Medicine, İstanbul-*Türkiye*

OBJECTIVE

Endoplasmic reticulum (ER) stress, which occurs as a result of the accumulation of misfolded or unfolded proteins, has been observed in many cancers. To re-establish the ER homeostasis, pathways of unfolded protein response (UPR) are activated. The effect of ER stress-activated UPR pathways on leukemogenesis has not been elucidated. In this study, we aimed to find out whether activated UPR pathways were involved in acute myeloid leukemia (AML).

METHODS

Expression levels of the 2 UPR components, binding immunoglobulin protein (BiP), and X-box protein 1 (XBP1), as well as the HLA-B were analyzed in 100 newly diagnosed AML patients using quantitative and qualitative polymerase chain reaction techniques.

RESULTS

Fifteen of the 100 newly diagnosed AML patients were shown to carry the spliced XBP1 (XBP1s) variant. Furthermore, in patients with acute promyelocytic leukemia (APL), a subtype of AML, expression levels of XBP1, and BiP were significantly higher than in non-APL patients ($p<0.001$ and $p<0.015$, respectively). It was determined that 6 of 15 (40%) AML patients carrying XBP1s variant were positive for myeloperoxidase.

CONCLUSION

Our study provides adequate evidences for the involvement of activated UPR pathways in some of the AML patients by showing the presence XBP1s variant and increased expression levels for XBP1 and BiP. Thus, agents targeting XBP1s might serve as a new approach for anti-cancer therapy.

Keywords: Acute myeloid leukemia; binding immunoglobulin protein; cancer; endoplasmic reticulum stress; X-box protein 1.

INTRODUCTION

Proteins are the principal actors of cellular function. Proper regulation of protein synthesis is utmost importance for the maintenance of cellular homeostasis and survival of cells in the presence of external and internal insults. Cancer leads to a number of cytotoxic conditions caused by tumor cells such as hypoxia, nutritional deficiency,

Received: February 26, 2023
Accepted: July 09, 2023
Online: August 02, 2023

Accessible online at:
www.onkder.org

Dr. Neslihan ABACI
İstanbul Üniversitesi,
Aziz Sancar Deneysel Tıp Enstitüsü,
Genetik Anabilim Dalı,
İstanbul-Türkiye
E-mail: neslihan.abaci@istanbul.edu.tr

and changes in pH, which results in the disruption of normal protein synthesis and accumulation of unfolded or misfolded proteins in the endoplasmic reticulum (ER), called ER stress. Cells have acquired mechanisms to cope with such aberrant protein synthesis and to re-establish the ER homeostasis. In tumor cells, various genetic, transcriptional, and metabolic abnormalities cause persistent ER stress. It creates adverse microenvironments affecting the functions, fate, and survival of cells.[1]

The unfolded protein response (UPR) is cellular stress response mechanism designed to reinstate correct protein folding and to prevent the accumulation of unfolded proteins in the ER lumen. Cancer cells stimulate UPR signaling pathways, consisting of three main tracks called protein kinase RNA-like ER kinase, activating transcription factor 6, and inositol-requiring enzyme 1 (IRE1), to overcome the ER stress.[2] Activation of IRE1α regulates several genes involved in ER homeostasis, cell survival, autophagy, apoptosis, angiogenesis, metastasis, drug resistance, and lipid metabolism.[3]

IRE1α cleaves X-box protein 1 (XBP1) mRNA when the cell enters into ER stress, resulting in the formation of XBP1s, a highly active transcription factor. The unspliced isoform is constitutively expressed and is called XBP1u.[4] The XBP1s involved in the UPR signal pathway, allow the survival of the ER stress in the cell by inducing genes included in ER chaperones and ER-related degradation mechanism.[5–7] In addition, ER chaperone protein (Binding immunoglobulin protein [BiP]/GRP78) is one of the most active components of cancer cells and has been reported to be overexpressed in many different types of cancer. In ER stress, BiP contributes to re-establishment of homeostasis through the correction of unfolded or misfolded proteins.[8]

XBP1s expression levels have been shown to increase in many cancers, such as multiple myeloma, breast cancer, hepatocellular carcinoma, and glioblastoma. This has been associated with cancer progression. There are reports stating increased expression of XBP1s in B-acute lymphoblastic leukemia (B-ALL) and in acute promyelocytic leukemia (APL) cells; in the latter, the PML-RARA fusion protein being responsible for the activation of UPR.[9,10] In addition, XBP1 has many functions in pre-B-ALL cells and activates pro-apoptotic pathways when the cell loses its vital survival and proliferation functions.[3]

Acute myeloid leukemia (AML) is a cancer of the hematopoietic system characterized by the malignant transformation of myeloid progenitor cells into blasts which interfere with normal hematopoiesis through accumulating in the bone marrow and peripheral blood.[11,12]

To investigate the role of UPR involvement in AML, we analyzed the expression levels of 2 UPR components, BiP and XBP1, as well as HLA-B, which is known to play a role in ER pathways and is discussed to be involved in the pathogenesis of AML.[13,14]

MATERIALS AND METHODS

Patient Samples

A total of 100 patients including 23 pediatric patients (12 female and 11 male) with diagnosed AML at Istanbul University Istanbul Faculty of Medicine, Division of pediatric hematology and oncology, and 77 adult (33 female and 44 male) patients with diagnosed AML at Istanbul University, Cerrahpaşa Medical Faculty, Haematology Department were included in this study. These patients are consecutive patients at diagnosis. Clinical and laboratory characteristics of the patients are presented in Table 1. In addition, bone marrow or peripheral blood samples were obtained from 8 of these 100 patients at 33rd day of treatment. Commercial total RNA samples (Clontech USA, cat no: 636591) (aged 22–85 years) were used as healthy control.

Quantitative and Qualitative Polymerase Chain Reaction (PCR)

RNA isolation from the AML samples was performed using PureLink, RNA Mini Kit (Invitrogen). cDNA synthesis from the RNAs of the resulting patient samples was performed using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche). cDNA synthesis conditions were adjusted to be 1 h at 37°C and 5 min at 94°C. Quantitative real-time PCR method was performed to determine the expression levels of XBP1, HLA-B, and BiP genes. LightCycler 480 instrument and Light Cycler 480 SYBR Green I kit were used for quantitation. Primers were designed for HLA-B and BiP genes, and the 18S rRNA gene was used as a reference gene. Primers designed specifically for XBP1 are capable of distinguishing between XBP1s and XBP1u. To observe the XBP1s variant, the qualitative PCR conditions were set at one cycle of 94°C for 2 min after preliminary denaturation, 35 cycles of 30 s at 94°C, 30 s at 57°C, 30 s at 72°C, and final extension for 5 min at 72°C. The primers used for XBP1 are as follows: Forward, 5'CCTTGTAGTTGAGAACCAGG-3', and reverse, 5'-GGGGCTTGGTATATATGTGG-3'. The primers generate two PCR products of 442 (XBP1u) and 416 (XBP1s) bps, respectively. PCR products were also run on a 3% agarose gel (Fig. 1).

Turk J Oncol 2023;38(4):488–95
doi: 10.5505/tjo.2023.3968

Table 1 Clinical and laboratory characteristics of AML patients

Characteristics	t(15;17) (n=16)	t(8;21) (n=12)	inv(16) (n=8)	FLT3 (n=2)	Other cases (n=62)
Age (years)					
0–17	8	4	2	–	9
18–85	8	8	6	2	53
Sex					
Male	7	6	4	1	37
Female	9	6	4	1	25
WBC (cells/mm³)	3438.5 (n=8)	33737.5 (n=5)	47180 (n=5)	NA	27933.20 (n=53)

AML: Acute myeloid leukemia; t: Translocation; inv: Inversion; FLT3: Fms-like tyrosine kinase 3; WBC: White blood cell; NA: Not available

Statistical Analysis

Data obtained in the study were statistically analyzed using the SPSS 16.0 Software (SPSS, Inc., Chicago, IL, USA). Independent sample t-test was used for comparing parametric data, and Kruskal–Wallis and Mann–Whitney U tests were used to compare non-parametric data. Correlation was studied using the Pearson and Spearman's tests. Chi-square test was used to compare ratios. P<0.05 was considered statistically significant.

RESULTS

In our study, XBP1s variant was found in 15 out of 100 patients with AML (%15) (Table 2). Two of these patients were carried t(15; 17), one patient was positive for inv(16), another one case carried FLT3-ITD mutation, and the rest 11 cases did not display any of these specific changes (Fig. 2).

Expression levels of XBP1, BiP, and HLA-B genes were analyzed to determine impact of the ER-mediated pathway and were compared with other clinical parameters in AML such as age, sex, and leukocyte count. There was no significant correlation in between sex (Appendix 1), age, and leukocyte count compared with XBP1, BiP, and HLA-B expressions (Table 3).

A significant correlation (p<0.001) was observed between BiP and XBP1 expressions. HLA-B and XBP1 expressions were not significantly correlated (p=0.14), while there was a robust correlation between the expression levels of HLA-B and BiP (p=0.019) (Table 3). When BiP and XBP1 expression levels were analyzed against the presence of t(15;17), t(8;21), inv(16), and FLT3-ITD mutations, BiP and XBP1 levels were found to be significantly higher in carriers of t(15;17) (p<0.001) and p=0.015, respectively)

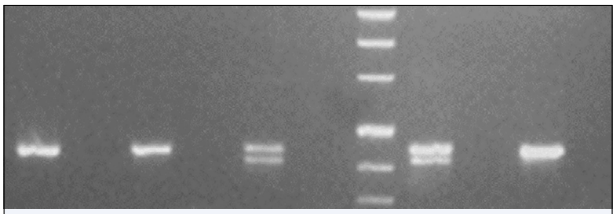


Fig. 1. Sample 1, 2, 5 are X-box protein 1 (XBP1) unsplicing variants (XBP1u) and 3, 4 XBP1 splicing variants (XBPs) variants in acute myeloid leukaemia cases.
M: Marker Puc Mix 8.

(Table 4) (Appendix 2). HLA-B and BiP expression levels did not correlate significantly among XBP1 expression (p=0.52 and p=0.19, respectively) (Table 3).

Furthermore, XBP1u expression was found higher than XBP1s between patients with XBP1s variants and without XBP1s variants (p=0.003). No significant results were found when age, gender, leukocyte count, and blast rate were compared statistically in XBP1s and XBP1u cases. The results are listed in Table 5. XBP1s variants in two AML patients with t(15;17) and one patient inv(16) were not observed in the 33rd day samples of the patients, although they were still positive for both t(15; 17) and inv(16). When the patients carrying the XBP1s variant were examined, 6 out of 15 patients were found to be myeloperoxidase (MPO) positive patients.

AML is a malignant disease that is caused by uncontrolled clonal proliferation of precursor hematopoietic cells. While several genetic and environmental factors have been accused to involve in the pathogenesis of AML, the etiology is not fully understood yet.[11,12] Earlier studies have demonstrated an association between cancer and ER stress components.[15] Knowing this we analyzed the expression levels of 2 important UPR components, XBP1 and BiP genes, in patients with AML.

Table 2 Distribution of XBP1 variants in AML cases

Genetic abnormalities	XBP1s (+) (n=15)		XBP1s (–) (n=85)	
	n	%	n	%
t(15;17)	2	13.3	16	18.8
t(8;21)	-	-	12	14.2
inv(16)	1	6.7	7	8.2
FLT3-ITD	1	6.7	1	1.2
No mutation	11	73.3	49	57.6
Total	15		85	

XBP1: X-box protein 1; AML: Acute myeloid leukemia; t: Translocation; inv: Inversion; FLT3: Fms-like tyrosine kinase 3

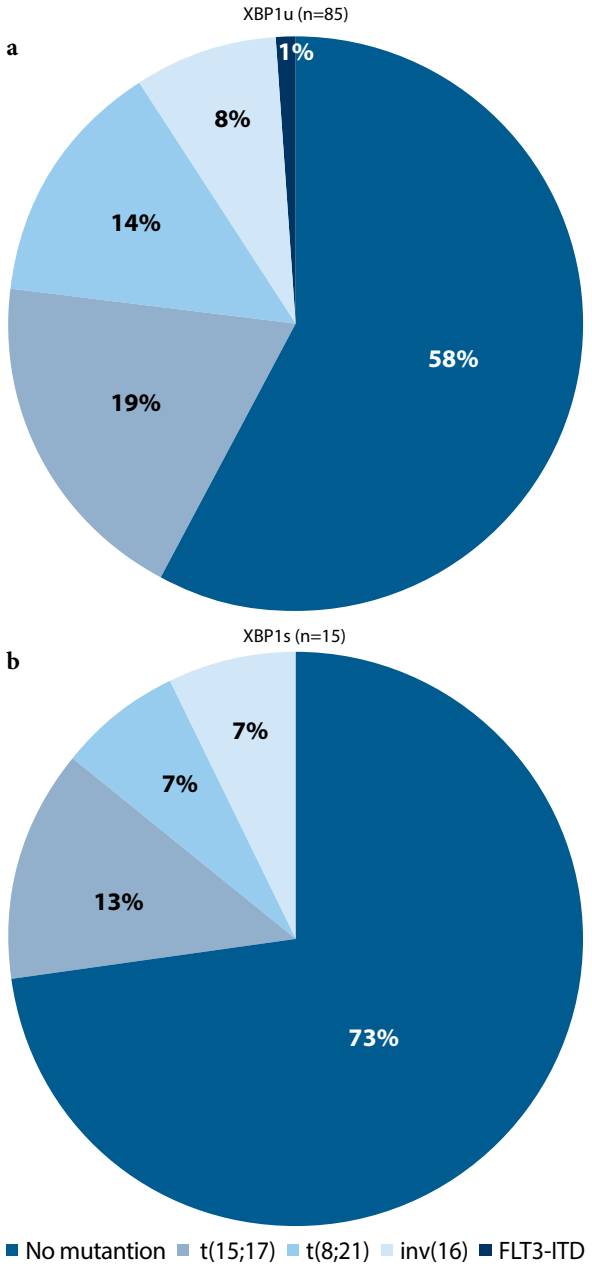


Fig. 2. Pie chart of distribution of X-box protein 1 (XBP1) variants in acute myeloid leukemia (AML) patients. (a) Distribution of XBP1u variants in AML patients (b) Distribution of XBP1s variants in AML patients.
t: Translocation; inv: Inversion; FLT3-ITD: Fms-like tyrosine kinase 3 internal tandem duplication

Mechanisms that activate the UPR in leukemia cells have not yet been fully disclosed. When the ER stress activates the UPR pathways, IRE1α cuts XBP1 and forms the XBP1s variant. Our results of XBP1s variant rates (15%) which we easily detected by quantitative PCR is similar to those of Schardt et al. (16.2%) in AML patients. Unlike Schardt et al.,[15] however, we could identify XBP1s variant in patients with inv(16). Our results confirm the findings of the latter study and serve as preliminary evidence for the relationship between AML and UPR. Expression levels of BiP and XBP1s involved in UPR were observed to be higher in the t(15;17) positive patients. This positive correlation between BiP and XBP1 levels indicates that UPR pathways are active in APL. Vice versa HLA-B expression levels were not significantly elevated in patients with AML. When proteins are improperly folded or unfolded in the cell, they tend to accumulate and activate the ER stress pathways.[7] MPO expression, as measured by cytochemistry, in leukemia blasts is widely accepted as a gold marker for the diagnosis of AML. Expression of MPO provides critical information regarding the phenotype of AML cells.[16]

We thought that, in patients with high MPO levels, ER stress pathways could be highly stimulated due to the accumulation of these MPO granules. There was an increase in the MPO levels in the 6 AML cases, in which found the XBP1s variant.

This finding suggests that the accumulation of MPO can trigger ER stress and activate UPR pathways, especially IRE1-XBP1 pathway. In the Ph + ALL subclasses, imatinib treatment reduced XBP1 expression.[9] Since XBP1 is more associated with survival, XBP1 expression is also decreased when cell apoptosis is induced by bortezomib treatment in patients with multiple myeloma.[17] In our cohort, we could show the disappearance of XBP1s variants in 2 patients with t(15;17) and one with inv(16) positivity. This indicates that anti-cancer treatment might induce XBP1 cleavage by activating UPR,[8] but that XBP1 expression is likely to decrease as different UPR pathways can be selected. The unsignificant results might be related to low cohort number. We hope that this study will be forward for other research.

DISCUSSION

AML is a malignant disease that is caused by uncontrolled clonal proliferation of precursor hematopoietic cells. While several genetic and environmental factors have been accused to involve in the pathogenesis of AML, the etiology is not fully understood yet.[11,12] Earlier studies have demonstrated an association between cancer and ER stress components.[15] Knowing this we analyzed the expression levels of 2 important UPR components, XBP1 and BiP genes, in patients with AML. Mechanisms that activate the UPR in leu-

Turk J Oncol 2023;38(4):488–95
doi: 10.5505/tjo.2023.3968

Table 3 Correlation analysis between HLA-B, BiP, XBP1 expressions and patient leukocyte count and ages

	Correlation	HLA-B	BiP	XBP1	Leukocyte	Age
HLA-B	r	1	0.235	0.151	–0.178	0.108
	p	-	**0.019**	0.133	0.124	0.283
	n	100	100	100	76	100
BiP	r	0.235	1	0.529	–0.126	–0.102
	p	**0.019**	–	**<0.001**	0.280	0.315
	n	100	100	100	76	100
XBP1	r	0.151	0.529	1	0.004	–0.080
	p	0.133	**<0.001**	–	0.976	0.429
	n	100	100	100	76	100
Leukocyte	r	–0.178	–0.126	0.004	1	0.106
	p	0.124	0.280	0.976		0.361
	n	76	76	76	76	76
Age	r	0.108	–0.102	–0.08	0.106	1
	p	0.283	0.315	0.429	0.361	
	n	100	100	100	100	76

HLA-B: Human leukocyte antigen-B; BiP: Binding immunoglobulin protein; XBP1: X-box protein 1; r: Correlation factor; p: Statistical significance, n: Number

Table 4 Comparison of HLA-BiP and XBP1 expressions between t(15;17), t(8;21), inv(16), and FLT3 positive and negative cases

	n	HLA-B		BiP		XBP1	
		Average	p	Average	p	Average	p
t(15;17)							
Positive	16	0.917	0.579	0.279	**<0.001**	0.054	**0.015**
Negative	84	0.708		0.139		0.036	
t(8;21)							
Positive	12	0.436	0.107	0.146	0.865	0.050	0.815
Negative	88	0.783		0.164		0.037	
inv(16)							
Positive	8	0.684	0.839	0.129	0.770	0.036	0.694
Negative	92	0.747		0.164		0.039	
FLT3							
Positive	2	0.398	0.414	0.080		0.031	
Negative	98	0.749		0.163		0.039	

HLA-BiP: Human leukocyte antigen- binding immunoglobulin protein; XBP1: X-box protein 1; t: Translocation; inv: Inversion ; FLT3: Fms-like tyrosine kinase 3; HLB-B: Human leukocyte antigen-B; BiP: Binding immunoglobulin protein; n: Number; p: Statistical significance

kemia cells have not yet been fully disclosed. When the ER stress activates the UPR pathways, IRE1α cuts XBP1 and forms the XBP1s variant. Our results of XBP1s variant rates (15%) which we easily detected by quantitative PCR is similar to those of Schardt et al. (16.2%) in AML patients. Unlike Schardt et al.,[15] however, we could identify XBP1s variant in patients with inv(16). Our results confirm the findings of the latter study and serve as preliminary evidence for the relationship between AML and UPR. Expression levels of BiP and XBP1s involved in UPR were observed to be higher in the t(15;17) positive patients. This positive correlation between BiP and XBP1 levels indicates that UPR pathways are active in APL. Vice versa HLA-B expression levels were not significantly elevated in patients with AML. When proteins are improperly folded or unfolded in the cell, they tend to accumulate and activate the ER stress pathways.[7] MPO expression, as measured by cytochemistry, in leukemia blasts is widely accepted as a gold marker for the diagnosis of AML. Expression of MPO provides critical information regarding the phenotype of AML cells.[16] We thought that, in patients with high MPO levels, ER stress pathways could be highly stimulated due to the accumula-

Table 5 Comparison of age, sex, blast, and leukocyte values of AML patients with XBP1u and XBP1s

	XBP1s n=15	XBP1u n=85	p
Sex			
Female	6	39	
Male	9	46	
Average of age	40.33 (8–80)	43.86 (1–85)	0.882
Mean of leukocyte count	34784.62	27037.30	0.091
Blast rate average	71.86	48.16	
M0	–	4	
M1	–	4	
M2	1	11	
M3	2	14	
M4	6	10	
M5	–	4	
M6	–	2	
M7	–	2	

AML: Acute myeloid leukemia; XBP1: X-box protein 1; n: number; p: Statistical significance

tion of these MPO granules. There was an increase in the MPO levels in the 6 AML cases, in which found the XBP1s variant. This finding suggests that the accumulation of MPO can trigger ER stress and activate UPR pathways, especially IRE1-XBP1 pathway.

In the Ph + ALL subclasses, imatinib treatment reduced XBP1 expression.[9] Since XBP1 is more associated with survival, XBP1 expression is also decreased when cell apoptosis is induced by bortezomib treatment in patients with multiple myeloma.[17] In our cohort, we could show the disappearance of XBP1s variants in 2 patients with t(15;17) and one with inv(16) positivity. This indicates that anti-cancer treatment might induce XBP1 cleavage by activating UPR,[8] but that XBP1 expression is likely to decrease as different UPR pathways can be selected. The unsignificant results might be related to low cohort number. We hope that this study will be forward for other research.

CONCLUSION

In this study, we have found that pathways of UPR are active in t(15;17)-positive AML, and we have shown that these pathways may be related to AML pathogenesis. In ER stress, XBP1 involves a strong UPR pathway more associated with cell survival.[18] Our findings indicate that XBP1 might play a facilitating role in the pathogenesis of AML. The mechanism of function of XBP1 in setting of AML is an area of interest for developing new target specific therapeutic strategies which involve UPR. Therapeutic approaches targeting UPR in cancer includes enhancing misfolded protein synthesis in ER to overload the UPR which results in more severe ER stress to cause cell death or inhibiting UPR adaptive and survival pathways for increasing susceptibility to anticancer therapy.[8,19]

Peer-review: Externally peer-reviewed.

Conflict of Interest: All authors declared no conflict of interest.

Ethics Committee Approval: The study was approved by the Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (no: 21, date: 22/12/2017).

Financial Support: The project was supported by the Scientific Research Projects Coordination Unit of Istanbul University (Project number: 574).

Authorship contributions: Concept – N.A., N.E.B., Ş.P.; Design – N.A., N.E.B., Z.E.; Supervision – N.A., S.S.E.; Funding – N.A.; Materials – M.C.A., A.Ü., T.E., A.S., Z.K., Ş.P.; Data collection and/or processing – M.C.A., A.Ü., T.E., A.S., Z.K.; Data analysis and/or interpretation – N.E.B., Ş.P., S.S.E., Z.E.; Literature search – M.C.A., A.Ü., T.E., A.S., Z.K., Ş.P., Z.E.; Writing – N.A., N.E.B., Ş.P., M.C.A.; Critical review – S.S.E., M.C.A., A.Ü.

REFERENCES

1. Chen X, Cubillos-Ruiz JR. Endoplasmic reticulum stress signals in the tumour and its microenvironment. Nat Rev Cancer 2021;21(2):71–88.
2. Cubillos-Ruiz JR, Bettigole SE, Glimcher LH. Tumorigenic and Immunosuppressive Effects of Endoplasmic Reticulum Stress in Cancer. Cell 2017;168(4):692–706.
3. Chen S, Chen J, Hua X, Sun Y, Cui R, Sha J, et al. The emerging role of XBP1 in cancer. Biomed Pharmacother 2020;127:110069.
4. Xu W, Wang C, Hua J. X-box binding protein 1 (XBP1) function in diseases. Cell Biol Int 2021;45(4):731–9.
5. Glimcher LH. XBP1: the last two decades. Ann Rheum Dis 2010;69(Suppl 1):i67–71.
6. Jiang D, Niwa M, Koong AC. Targeting the IRE1α–XBP1 branch of the unfolded protein response in human diseases. Semin Cancer Biol 2015;3:48–56.
7. Ma JH, Wang JJ, Zhang SX. The unfolded protein response and diabetic retinopathy. J Diabetes Res 2014;2014:160140.
8. Yadav RK, Chae SW, Kim HR, Chae HJ. Endoplasmic reticulum stress and cancer. J Cancer Prev 2014;19(2):75–88.

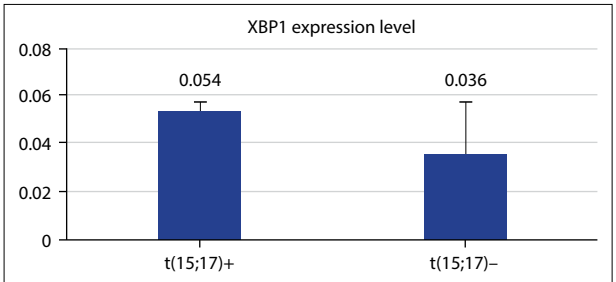
Turk J Oncol 2023;38(4):488–95
doi: 10.5505/tjo.2023.3968

9. Kharabi Masouleh B, Chevet E, Panse J, Jost E, O'Dwyer M, Bruemmendorf TH, et al. Drugging the unfolded protein response in acute leukemias. J Hematol Oncol 2015;16;8:87.
10. Chevet E, Hetz C, Samali A. Endoplasmic reticulum stress-activated cell reprogramming in oncogenesis. Cancer Discov 2015;5(6):586–97.
11. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. N Engl J Med 2015;373(12):1136–52.
12. Zwaan CM, Kolb EA, Reinhardt D, Abrahamsson J, Adachi S, Aplenc R, et al. Collaborative efforts driving progress in pediatric acute myeloid leukemia. J Clin Oncol 2015;33(27):2949–62.
13. Smith JA, Turner MJ, DeLay ML, Klenk EI, Sowders DP, Colbert RA. Endoplasmic reticulum stress and the unfolded protein response are linked to synergistic IFN-beta induction via X-box binding protein 1. Eur J Immunol 2008;38(5):1194–203
14. Fernández-Torres J, Flores-Jiménez D, Arroyo-Pérez A, Granados J, López-Reyes A. HLA-B*40 allele plays a role in the development of acute leukemia in Mexican population: a case-control study. Biomed Res Int 2013;2013:705862.
15. Schardt JA, Weber D, Eyholzer M, Mueller BU, Pabst T. Activation of the unfolded protein response is associated with favorable prognosis in acute myeloid leukemia. Clin Cancer Res 2009;15(11):3834–41.
16. Itonaga H, Imanishi D, Wong YF. Expression of myeloperoxidase in acute myeloid leukemia blasts mirrors the distinct DNA methylation pattern involving the downregulation of DNA methyltransferase DNMT3B. Leukemia 2014;28:1459–66.
17. Vincenz L, Jäger R, O'Dwyer M, Samali A. Endoplasmic reticulum stress and the unfolded protein response: targeting the Achilles heel of multiple myeloma. Mol Cancer Ther 2013;12(6):831–43.
18. Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat Rev Mol Cell Biol 2012;13(2):89–102.
19. Li X, Zhang K, Li Z. Unfolded protein response in cancer: the physician's perspective. J Hematol Oncol 2011;4:8.

APPENDIX 1 Correlation between HLA-B, BiP, XBP1 genes and gender in AML patients

	Sex	n	Average	SD	p
HLA-B	Female	45	0.720	0.8181	0.366
	Male	55	0.760	0.6149	
BiP	Female	45	0.169	0.1266	0.615
	Male	55	0.155	0.1419	
XBP1	Female	45	0.041	0.0430	0.737
	Male	55	0.037	0.0369	

HLA-B: Human leukocyte antigen-B; BiP: Binding immunoglobulin protein; XBP1: X-box protein 1; n: Number; SD: Standard deviation; p: Statistical significance



APPENDIX 2. XBP1 gene expression level in t(15;17) positive and negative AML patients.
XBP1: X-box protein 1; t: Translocation; AML: Acute myeloid leukemia