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The Evaluation of the Role of Several Genes (BUB1, BUB1B, and TOP2A) and the Related Signaling Pathway in the Pathogenesis of Acute Lymphocytic Leukemia (ALL): Combination of in Silico and Experimental Analysis

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is one of the most common cancers in children, which causes the death of many patients. Nowadays, bioinformatics approaches are widely used to identify factors involved in the pathogenesis of diseases. Given the extent of molecular pathways and genes involved in these pathways, the use of bioinformatics approaches and the experimental validation of genes is essential. In this regard, we evaluated the roles of TOP2A, BUB1, and BUB1B genes in the biological processes of ALL, as well as their expression in malignant ALL cells.

Materials and Methods: The present work is a bioinformatics and experimental study. The GSE102301 database was used in this study. After determining Differentially Expressed Genes (DEGs), cell lines were used to evaluate gene expression. The cell line used in this study was CCRF-CEM. The expression of TOP2A, BUB1, and BUB1B genes for validation was evaluated using real-time polymerase chain reaction (RT-PCR).

Results: In this study, 72 DEGs were identified in ALL patients compared to the control subjects. Out of 4234 DEGs, 35 genes were up-regulated and 37 genes were down-regulated. The expression of BUB1, BUB1B, and TOP2A was increased in CCRF-CEM cells compared to peripheral blood mononuclear cells (PBMCs). The difference in the expression of these genes between the two cell types was statistically significant ($p < 0.05$).

Conclusion: The findings suggest that BUB1, BUB1B, and TOP2A are significantly overexpressed in ALL cells and may play a role in the disease's pathogenesis. These genes warrant further investigation as potential biomarkers and prognostic indicators in ALL, especially in clinical patient cohorts.

Keywords: Acute Lymphocytic Leukemia, Bioinformatics, Topoisomerase

Introduction

Acute lymphoblastic leukemia (ALL) is one of the most common cancers in children, which causes the death of many patients (1). The average age of ALL diagnosis in patients is 15 years. ALL is characterized by dysfunction in the differentiation of lymphoid cells in the bone marrow and peripheral blood (2). In this malignancy, the function and development of blood lymphocytes are disturbed. According to statistical evidence, 6000 new cases are diagnosed annually and 1500 patients die from the

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disease (1, 3). Pathogenesis of ALL is a combination of genetics, environmental factors, and biology. Based on molecular methods, including next-generation sequencing (NGS), it has been determined that mutation, transfer, deletion, and genetic abnormalities play an important role in the occurrence and progression of ALL (4, 5). On the other hand, gene expression disorders are also influential factors in ALL. Disruption of gene expression activates or deactivates signaling pathways. Signaling pathways are involved in regulating cellular biological activities including proliferation, apoptosis, and cell communication. Increased proliferation or inhibition of apoptosis is one of the indicators of resistance to treatment in ALL patients (6-9).

Based on systems biology approaches, it has been shown that the interactions between genes and molecular pathways can largely indicate the pathogenesis of diseases. There are a series of genes that are known as hub genes due to their increased interactions with other genes (10-13). In the present study, after a bioinformatics analysis, three hub genes were identified in ALL: Topoisomerase II, which has two genetically distinct isoforms (TOP2A), budding uninhibited by benzimidazoles (BUB1), and BUB1B. TOP2A is one of the genes whose expression in cells is accompanied by increased proliferation. The results showed that TOP2A expression in cancer cells can be associated with cell proliferation and the inhibition of apoptosis (14).

BUB1 is one of the genes that is part of a cell structure. Evidence shows that BUB1 contributes to the regulation of chromatin and chromosomes. Disruption of BUB1 function or expression can cause dysregulation of biological processes in the cell (15).

Nowadays, bioinformatics approaches are widely used to identify factors involved in the pathogenesis of diseases. Given the extent of molecular pathways and genes involved in these pathways, the use of bioinformatics approaches and the experimental validation of genes is essential. In this regard, we evaluated the roles of TOP2A, BUB1, and BUB1B genes in the biological processes of ALL, as well as their expression in malignant ALL cells.

Materials and Methods

Characteristics of data

In this study, the database GSE102301 extracted from Gene Expression Omnibus (GEO) was used. The platform of GSE102301 was GPL16791 Illumina HiSeq 2500 (Homo sapiens). R software was applied to determine Differentially Expressed Genes (DEGs). The criteria used included $|logFC| > 2$ and $p < 0.05$.

Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway

GeneCodis4 software was used for GO analysis related to DEGs. For this purpose, molecular functions (MF), cellular components (CC), and biological processes (BP) related to DEGs were identified. Moreover, GeneCodis4 software was used to analyze the KEGG to determine the related molecular pathways. $p < 0.05$ was determined as a significance level for the analyses.

Designing the protein-protein interaction (PPI)

PPI was used to evaluate the interactions and networks between proteins and DEGs. STRING database was employed to design and specify the interactions between proteins. Cytoscape software was used to observe these interactions. For PPI analysis, a minimum required interaction score > 0.4 was utilized.

Cell culture

In this study, the CCRF-CEM cell line was purchased from the Pasteur Institute of Iran. The cells were cultured in Dulbecco's

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modified Eagle medium (DMEM)/F12 supplemented with 10% fetal bovine serum (FBS) (Cytomatingene, Iran) and 1% penicillin-streptomycin (Cytomatingene, Iran). The cells were maintained in a humidified incubator at 37°C with 5% CO_2. Peripheral blood mononuclear cells (PBMCs) were used as control cells.

RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

To extract RNA from cells, the TRIzol reagent was used according to Marbateh's instructions. After RNA extraction, cDNA was synthesized using the TaqMan MicroRNA Reverse Transcription Kit and qRT-PCR was performed using the TaqMan MicroRNA Assay. The expression levels of TOP2A, BUB1, and BUB1B genes were normalized to U6 small nuclear RNA.

Results

Identification of DEGs

In this study, 72 DEGs were identified in ALL patients compared to the control subjects. Out of 4234 DEGs, 35 genes were up-regulated and 37 genes were down-regulated (Figure 1).

GO and KEGG enrichment analyses

GO analysis was conducted based on CC, MF, and BP. It was found that CC mainly played a role in the plasma membrane, granules, DNA replication, cell cycle, and hemoglobin complex; MF was shown to be involved in immune responses, cytokine secretion, and lipid metabolism; and BP mainly contributed to immune responses, cell differentiation, and antigen processing and presentation (Figure 2).

Determination of hub Genes

The PPI network depicted in the figure demonstrates a diverse array of interactions among various proteins, with notable central nodes such as FN1 and COL1A1, indicating their significant roles in cellular processes. The network reveals clusters of proteins that may collaborate in specific biological functions, such as extracellular matrix formation and cellular signaling. Interactions among proteins like VIM, LRP1, and MCP suggest potential pathways involved in cell adhesion and migration. This intricate web of connections not only highlights the complexity of protein interactions but also provides insights into the molecular mechanisms underlying various physiological and pathological conditions, presenting opportunities for further investigation into targeted therapeutic strategies (Figure 3).

Evaluation of Gene Expression

The results showed that the expression levels of BUB1, BUB1B, and TOP2A were increased in CCRF-CEM cells compared to PBMCs. The difference in the expression of these genes between the two cell lines was statistically significant ($p < 0.05$) (Figure 4).

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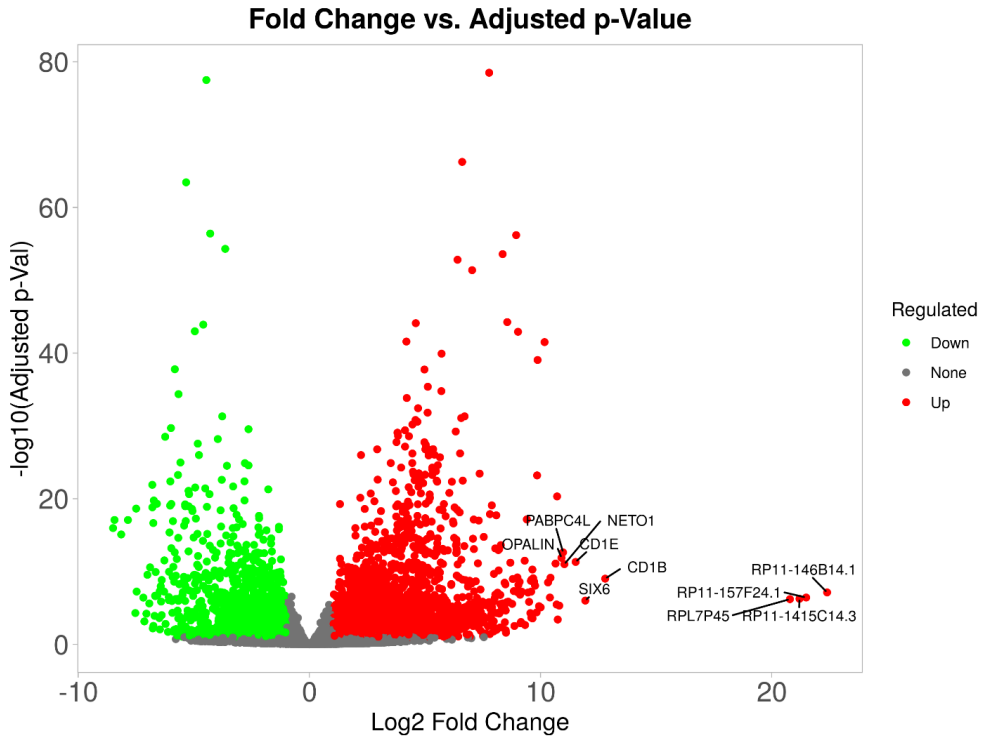

Figure 1. Volcano diagram-related DEGs. Volcano plot of DEGs identified from the GSE102301 dataset. The x-axis represents the log2 fold change (log2FC), and the y-axis shows the -log10 adjusted p-value. Genes with |log2FC| > 2 and $p < 0.05$ were considered statistically different. Up-regulated genes are shown in red, down-regulated genes in green, and non-significant genes in gray.

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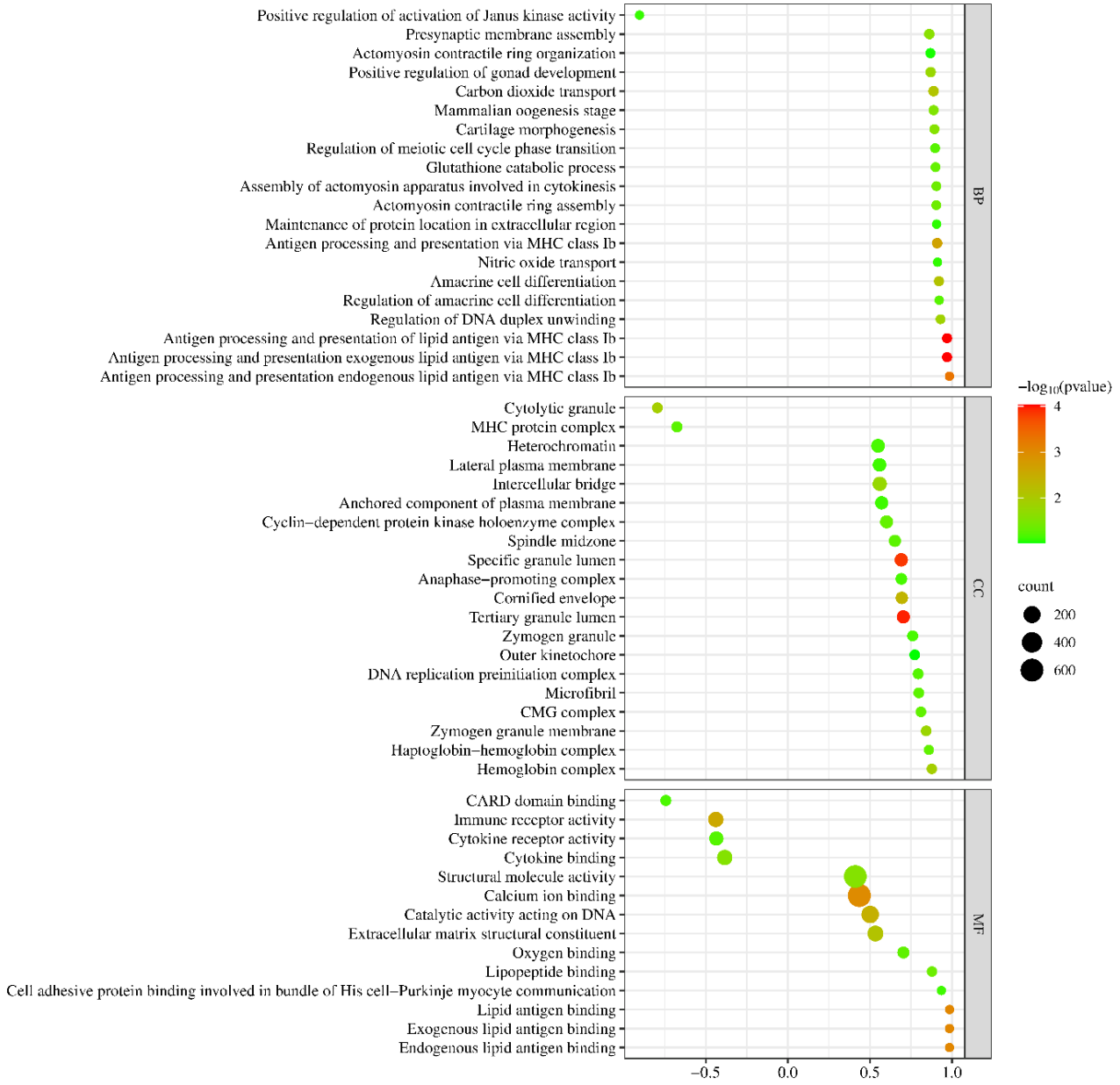

Figure 2. GO-related DEGs. GO-related BP, CC, and MF. For each section, the results showed pathways in cellular physiology.

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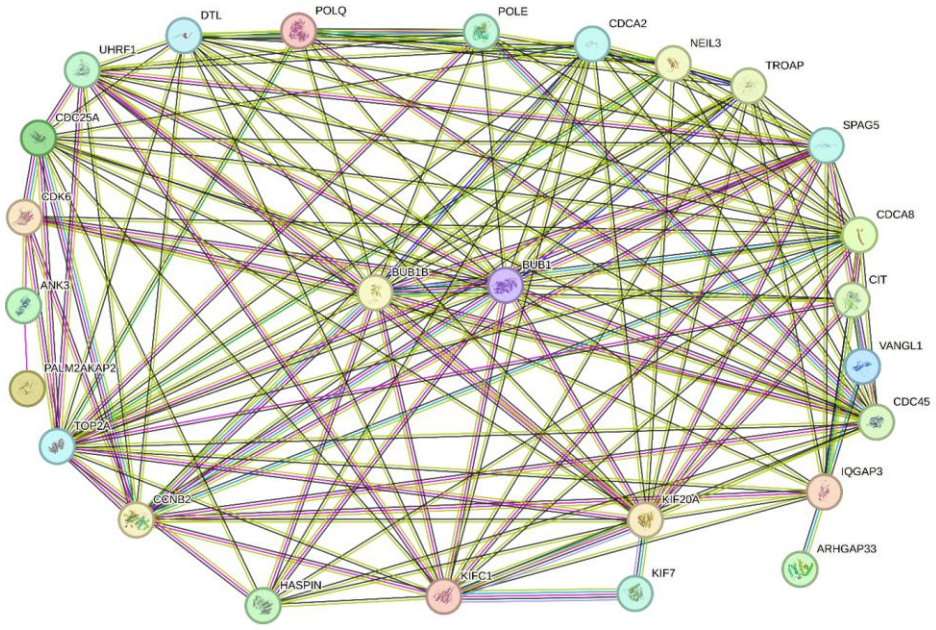

Figure 3. PPIs between DEGs. PPIs are designed by STRING and Cytoscape. Nodes represent proteins encoded by DEGs, and edges represent functional interactions. Hub GGenes such as FN1 and COL1A1 were identified based on degree centrality.

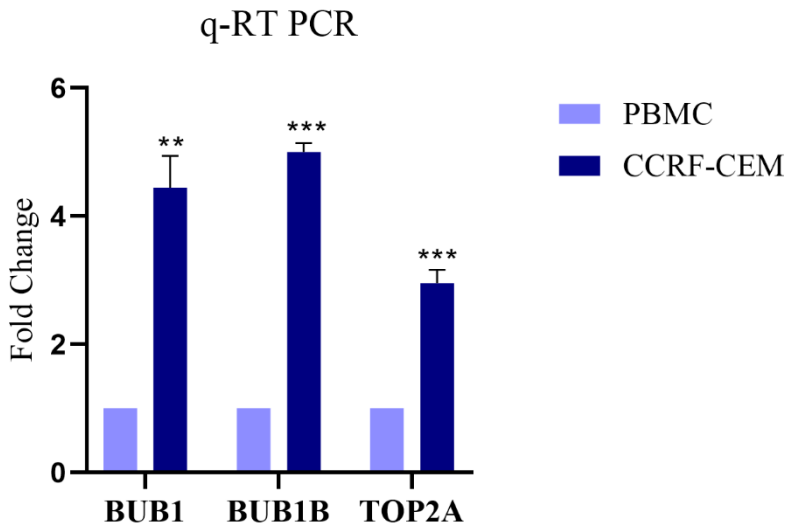

Figure 4. Gene expression of BUB1, BUB1B, and TOP2A in CCRF-CEM cells. The expression of all three genes was increased in CCRF-CEM cells compared to PBMCs. This increase in expression between the two groups was statistically significant (p = 0.01 for BUB1, p = 0.001 for BUB1B and TOP2A).

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Discussion

According to the results of the present study, in the in silico analysis, the BUB1, BUB1B, and TOP2A genes were identified as hub genes in ALL patients. In the experimental section, the results showed that the expression of these three genes was significantly increased in the CCRF-CEM cell line, which is known as ALL cells, compared to the control PBMCs ($p < 0.05$).

In line with the present study, Ren et al. demonstrated that TOP2A expression was increased in ALL samples. Their results showed that based on the function of TOP2A in chromatid separation and chromosome assembly, its expression is increased during cell mitosis. This increased expression leads to cell proliferation and prevents cell differentiation. Therefore, targeting TOP2A with chemotherapeutic drugs could partially prevent the progression of ALL (16). Another study also reported that TOP2A gene expression was elevated in patients with newly diagnosed ALL, though this increase in expression was not observed in a small number of cases. Further studies showed that glucocorticoid resistance was observed in patients without increased TOP2A expression (17). Tamaichi et al. indicated that mutations in ataxia telangiectasia mutated (ATM) can be associated with reduced binding to TOP2A. This reduction is associated with the sensitivity of ALL cells to TOP2 inhibitors. This sensitivity can be linked to mixed lineage leukemia (MLL) in patients. Conversely, the absence of mutations in ATM was associated with increased binding to TOP2A and its phosphorylation and ultimately insensitivity to TOP2 inhibitors (18). On the other hand, it has been shown that increased TOP2A expression can be associated with drug resistance in patients (19). Raghidbaghan et al. showed that vincristine reduces TOP2A expression through apoptosis. Their evaluations revealed that TOP2A controls the cell cycle and proliferation of ALL cells through interaction with cyclin-dependent kinase 1 (CDK1) (20).

In general, evidence suggests that increased TOP2A expression in patients might be associated with cell proliferation and insensitivity to chemotherapy. Therefore, targeting TOP2A can prevent disease progression and improve the clinical conditions of patients. Regarding BUB1, previous studies have shown that its increased expression is associated with poor prognosis in ALL patients. Consequently, findings have indicated that increased BUB1 expression can be associated with a reduced response to chemotherapy in patients. BUB1 causes cell growth and proliferation and resistance to chemotherapy drugs by increasing the cell cycle rate (21, 22). On the other hand, it has been shown that BUB1 is a target of the transcription factor T cell leukemia homeobox 1 (TLX1), which increases its expression. Increased expression of BUB1 and TLX1 in ALL patients leads to elevated cell proliferation and the inhibition of cell differentiation Moreover, BUB1 has been shown to play a role in chromosome aberrations in ALL patients (24).

BUB1 is a spindle assembly checkpoint (SAC) gene that accurately leads to chromosome fidelity and the distribution of chromosomes and attaches to kinetochores during mitosis. Kinetochores not correctly attached to microtubules recruit Cdc20-dependent inhibitors, including BUBR1, BUB1, BUB2, BUB3, Mad1, and Mad2, and suppress anaphase-promoting complex (APC). Two cascades regulate APC: BUBR1-BUB3-Cdc20 and Mad2-Cdc20. During SAC arrest, Mad2, BUB3, and BUBR1 inhibit the APC/C pathway (25). If the BUB genes undergo mutations, improper distribution of chromosomes can cause aneuploidy in daughter cells (26, 27). It was shown that BUB genes can suppress B cells. BUB1

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and BUB1B suppress immune responses by raising the secretion of the immune system's checkpoint molecules, which allows tumor cells to evade the immune system (28).

Conclusion

The findings suggest that BUB1, BUB1B, and TOP2A are significantly overexpressed in ALL cells and may play a role in the disease's pathogenesis. These genes warrant further investigation as potential biomarkers and prognostic indicators in ALL, especially in clinical patient cohorts.

Data availability

Data availability is the corresponding author's responsibility.

Ethical Considerations

Ethical code: IR.IUMS.REC.1402.179.

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Authors' Contributions

Nastaran Khodakarim designed the study. Maedeh Mohammadi, Azadeh Fateh, Mahya Sadat Yassini, Samane Rezapour drafted the manuscript. Ali Asghar Kiani conducted the data analysis. Bahareh Shateri Amiri conduct data collection.

Funding

Not applicable.

Conflict of Interest

Not applicable.

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