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High *TUBB2A* expression in childhood T-ALL is correlated with the clinical outcome

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Abstract

Introduction: Microtubules are polymers that perform functions such as mitosis, intracellular transport, cell morphology, and ciliary and flagellar motility. Since microtubules are taking active part in cell division, they are among direct targets of several antimitotic drugs.

Methods: Expression levels of tubulin isotypes were analyzed in microarray data of childhood diagnostic T-ALL samples (n = 31) and healthy thymocytes (n = 7). Findings were validated with qPCR in separate T-ALL cohort (n = 48), and clinical correlation analyses were performed. *TUBB2A*'s effects were tested with siRNA-mediated knockdown in MOLT4 cell line, and apoptosis assay was carried out at 24, 48, and 72 hours time points.

Results: In microarray data, *TUBB2A* was found to be the only differentially expressed tubulin isotype (adj. *P* value = .01), which was validated by qPCR (*P* = .02). Samples representing differentiation stages of T cell showed an increasing trend of *TUBB2A* toward mature T-cell stage. *TUBB2A* expression was significantly higher in high-risk group patients (*P* = .026) and in a group with WBC counts >100 ($\times 10^9$cells/L) (*P* = .029). High *TUBB2A* was also found to be a predictor of shorter OS (*P* = .029) and RFS (*P* = .042).

Conclusion: Aberrant expression of TUBB isotypes can affect the balance of microtubules or microtubule-associated proteins, which might lead to drug resistance/relapse. Contribution of cytoskeleton proteins to drug resistance needs further investigation, and understanding aberrant expression and mode of action of microtubules will improve therapy strategies.

KEYWORDS

leukemia, microarray, T-ALL, *TUBB2A*, tubulin

1 | INTRODUCTION

T-ALL develops as a result of acquired genetic and epigenetic changes in an immature thymocyte.[1] Several aberrations involved in cell cycle pathway activation (eg, NOTCH1 activation), cell cycle control (eg, *CDKN2* locus mutations), upregulation of transcription factors normally expressed (eg, MYC) and not expressed (eg, *TAL1*, *TAL2*) in thymocytes, loss-of-function mutation of transcription

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factor (eg, *LEF1*, *ETV6*, *BCL11B*, *RUNX1*, *GATA3*), mutations in chromatin remodeling proteins (*EZH2*, *SUZ12*, *PHF6*), and signal transduction proteins (*PTEN*, *ABL1*, *RAS*, *JAK2*, *JAK3*, *FLT3*, *IL7R*) were reported with different frequencies in T-ALL patients (for a review, see Ref. [2]).

Although 5-year survival rate of pediatric T-ALL patients now exceeds 85%, approximately 15%-20% of patients relapse after treatment and have poor prognosis, and 10-year relapse-free survival (RFS) is around 35%.[3,4] There are several proposed biomarkers predicting ALL prognosis such as age at diagnosis,[5] cytogenetic profile,[6] immunophenotype,[7] or induction failure.[8] T-ALL appears to be more complex and heterogeneous than B-ALL, and there is a need for biomarkers to better stratify the disease.

Microtubules are polymers that perform many different cellular functions such as mitosis, intracellular transport, asymmetric morphology of a cell, and ciliary and flagellar motility.[9] Microtubules are made up of α- and β-tubulins; there are known seven β- and eight α-tubulins in mammals.[10] Different tubulin isotypes are encoded by different genes located on different loci and possess specific tissue and developmental distributions.[11] Several studies have demonstrated differing composition of tubulin isotypes in different tissue types and disease conditions.[9,12-14] Since microtubules are taking active part in cell division, they are among direct targets of several antimitotic drugs.[15] Genome-wide approaches have been valuable in identifying novel candidate genes and pathways that might mediate drug sensitivity and/or resistance. Recent evidence has highlighted that different regulations of cytoskeleton proteins can mediate sensitivity to tubulin-binding drugs.

Reanalysis of our previous expression microarray study led us to TUBB gene family. In this study, we detected high expression of TUBB genes, specifically *TUBB2A* in T-ALL patients. We aimed to analyze possible clinical significance of *TUBB2A's* expression level in childhood T-ALL patients and found prognostic relevance to the *TUBB2A* expression and treatment response. Also, siRNA-mediated knockdown study was conducted to test whether the viability of T-ALL cells affected with downregulation of *TUBB2A*.

2 | MATERIALS AND METHODS

2.1 | Microarray data analysis

Microarray data were generated in our previous study[16] with accession number GSE46170 and used for differential gene expression analysis. This cohort consists of 7 healthy thymocyte subsets representing different T-cell developmental stages and 31 pediatric T-ALL samples taken at diagnosis. R 3.5.0 open source program and relevant packages were used for data processing, differential gene expression analysis, and plot generation (https://cran.r-project.org/). For quality control of expression data, *affyQC* module was used and T92 and T101 samples were detected to be the most divergent 2 samples in NUSE and RLE plots, so they were excluded from downstream analysis.

Raw Affymetrix CEL files were background-corrected and RMA-normalized with *affy package*. Differential gene expression was done using *limma* package where transcripts with at least 2 fold change and adj. *P* value < .05 were considered as having significant change. Heatmap and clustering plots were drawn using *gplots* package.

TUBB2A and other tubulin β's expression values and their prognostic relevance were also analyzed by PRECOG database (PREdiction of Clinical Outcomes from Genomic profiles), which measures every gene's prognostic relevance using expression microarray data and its corresponding survival information.[17] Database calculates *Z-scores* for every gene across different cancer types: positive *Z-scores* corresponding to the worse survival when gene is overexpressed and negative scores corresponding to better survival when gene is overexpressed.

2.2 | Patients and healthy controls

We studied a separate 48 childhood T-ALL patients to validate the microarray results obtained from 31 patients. The validation group were diagnosed between years 1997 and 2015 at pediatric hematology and oncology departments' clinics with immunophenotype positivity of CD3, CD5 and CD7 and bone marrow blast of >25%. Bone marrow samples of pediatric T-ALL patients at diagnosis with blast counts >90% were used for total RNA isolation. Clinical parameters (sex, age, white blood cell (WBC) count, hemoglobin (Hb), platelet (PLT), immune phenotype (EGIL classification), central nervous system (CNS) or other organ involvement, induction therapy response, relapse statuses, and last statuses) were evaluated with correlation studies (Table 1). Two T-ALL patients were carrying *TEL-AML* translocation, and 3 patients had *SIL-TAL* translocation. Pediatric patients were treated with BFM protocol according to the year of diagnosis, median overall survival was 44 months (min-max, 1-148), and relapse-free survival was 28 months (min-max, 0-148).

Healthy thymocyte subsets were sorted from thymus tissues obtained from open-heart surgeries of nonleukemic patients. Total thymus tissue (n = 6), CD3+ cells of thymus (n = 3), CD3− cells of thymus (n = 2), earliest precursor T cell—TN ($CD3^-CD4^-CD8^-$, triple negative, n = 2), DN ($CD4^-CD8^-$, double negative, n = 2), ISP ($CD8^+$, immature single positive, n = 2), SP4 (single CD4 positive, n = 4), and SP8 (single CD8 positive, n = 4), and $CD3^+$ peripheral blood (PB $CD3^+$, n = 2) were used as healthy controls. Following homogenization, the cells were stained according to the standard protocol[18] and sorted by FACSAria II. Written and oral informed consents were obtained from parents of patients, and study was approved by Ethical Committee of Istanbul University Faculty of Medicine (2008/305 and 2015/1821).

2.3 | Cell culture and siRNA treatment

To repress *TUBB2A*, we have checked *TUBB2A* expression levels in four different T-ALL cell lines (Molt4, Jurkat, Cem, and DND41) and

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TABLE 1 Clinical aspects of T-ALL cohort

T-ALL (n = 48)	
Age	
Median (min-max)	9 years (22days-16 years)
Sex (M:F)	3.3:1
WBC count at diagnosis (×10^9/L)	
Median (min-max)	77.800 (1.3-430.0)
Plt at diagnosis (×10^9/L)	
Median (min-max)	50 000 (11 300-310 000)
Hb (g/dL)	
Median (min-max)	9.3 (5.3 - 15.3)
Induction therapy response (day 33)	
Yes	34 (71%)
No	8 (17%)
NA	6 (12%)
Immune phenotype (EGIL classification)	
TI/II (immature)	16 (33%)
TIII (cortical)	17 (35%)
TIV (mature)	12 (25%)
NA	3 (6%)
CNS involvement	
Yes	15 (31%)
No	29 (60%)
NA	4 (9%)
Relapse	
Yes	11 (23%)
No	33 (69%)
NA	4 (8%)
Last status	
Ex	12 (25%)
Alive	31 (65%)
Out of follow-up	5 (10%)
Other organ involvement (n)	
Mediastinum	9 (19%)
Lymphadenopathy	20 (42%)
Splenomegaly	21 (44%)
Hepatomegaly	22 (46%)
Gallbladder	1 (2%)
Skeletal system	1 (2%)
Lung pleurisy	1 (2%)

Abbreviations: CNS, central nervous system; Hb, hemoglobin; NA, not available; Plt, platelet; WBC, white blood cells.

the highest expression was detected in Molt4. Molt4 cell line was grown in DMEM with 1% Pen/Strep and 10% FBS and incubated at 37°C with 5% CO_2 prior to siRNA transfection, and a pool composed of four predesigned siRNAs were used (Accell SMARTpool; Dharmacon, Inc) according to the manufacturer's instructions. Cells were seeded into 24-well plates with a number of 3 × 10^5 viable cells/well (200 μL), and transfected with 0.5 μM siRNA mix, and cells were collected at indicated time points (24, 48, and 72 hours). Experiment was carried out in duplicates. siRNA knockdown effectiveness was compared using mock (treated only with transfection reagent) as control.

2.4 | RNA isolation, cDNA synthesis, and Quantitative Real-time PCR (qRT-PCR)

Total RNA was isolated by Qiagen RNeasy Plus Mini Kit (Qiagen GmbH), and complementary DNA (cDNA) was synthesized by random hexamers and Moloney murine leukemia virus (MMLV) reverse transcriptase from 1 μg of total RNA according to the manufacturer's procedures (MBI Fermentas, Vilnius, Lithuania). Quantitative Real-time PCR was carried out on the Light Cycler 480 Instrument (Roche Applied Sciences, Manheim, Germany). *TUBB2A* was amplified with forward: CGACCCCACAGGCAGTTA and reverse: GGATGGCCCGAGGTACATA primers and UPL probe #78 (Roche diagnostics). For internal reference, peptidylprolyl isomerase A (*PPIA*) gene was used with forward: TATCTGCACTGCCAAGACTGAGTG and reverse: CTTCTTGCTGGTCTTGCCATTCC primers and UPL probe #48. Relative expressions were calculated according to the ΔΔCt method, based on the mathematical model as previously described.[19]

2.5 | Apoptosis assay

Cell viability was assessed with BD Biosciences Annexin V-FITC Kit and FACS cytometry (BD Biosciences) using cells collected at 3 time points (24, 48, and 72 hours after siRNA transfection) according to the manufacturer's protocol. $FITC^+PI^-$ cells were considered as apoptotic cells, $FITC^-PI^+$ as necrotic, and $FIRC^+PI^+$ cells as dead.

2.6 | Statistical analysis

For statistical analyses, R 3.5.0 was used in normalizing raw Affymetrix expression data, calculating fold change values and their respective *P* values. Relative expressions were compared by Student's *t* test between the groups. Overall survival (OS) was defined by the interval from the date of diagnosis to the date of death or last follow-up. Relapse-free survival (RFS) was the duration from the date of complete remission to last follow-up or to the first event (failure to achieve remission (early death or resistant leukemia), relapse or death in complete remission). The Kaplan-Meier method was used to estimate survival rates. $P \leq .05$ (two-sided) was considered statistically significant. Differences were compared with the two-sided log-rank test. All statistical analyses were done by SPSS for Windows 21.0 (IBM SPSS Data Editor Inc) and GraphPad 8 Software Inc.

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3 | RESULTS

3.1 | Expression array data reveal increased *TUBB2A* expression in pediatric T-ALL cohort

Differential gene expression analysis gave a list of 890 transcripts with at least twofold change and adj. *P* value < .05. *TUBB2A* was among the top 100 most significant genes (Table S1). The only significantly changed tubulin β isotype was *TUBB2A* with 5.7-fold upregulation and adj. *P* value = .01 in T-ALL compared with healthy isotype (Table S2). *TUBB6* also showed 2.2-fold upregulation in patients, but the *P* value was not significant (adj. *P* value = .2). As seen in Figure 1, tubulin β expression profile distinguishes almost perfectly between control and T-ALL samples. No significant expression changes were seen among tubulin α isotypes.

3.2 | High *TUBB2A* expression in normal and malignant conditions

Healthy thymocyte subsets were evaluated for *TUBB2A* expression levels. *TUBB2A expression* was gradually elevated toward maturation stages of T-cell development: *The* lowest expression was detected in TN stage and the highest was detected in SP4, SP8, and mature peripheral CD3+ T cells (Figure 2A). Also, T-ALL samples showed a significant increase in *TUBB2A* expression in T-ALL patients compared with healthy T cell subsets (*P* = .02, Figure 2B). Moreover, T-ALL patients were divided into their developmental stages according to EGIL classification and compared with their respective healthy T-cell controls. Immature T-ALL samples were seen to have the most prominent overexpression of *TUBB2A* (*P* = .009, Figure 2C) compared with its respective DN stage controls. Cortical and mature stage patients did not show significant difference compared with their stage-specific controls (*P* = .07 and .*75*, respectively).

3.3 | Clinical impact of increased *TUBB2A* expression in T-ALL patients

TUBB2A's expression levels were tested for their clinical correlation in T-ALL patients. Patients with WBC count above 100 ($\times 10^9$cells/L) had elevated *TUBB2A* expression levels than those with WBC counts below 100 ($\times 10^9$cells/L) (*P* = .029) (Figure S1A). WBC count was also found positively correlated with *TUBB2A* expression levels (Pearson correlation *r* = .36, *P* = .018, Figure S1B). Among the T-ALL risk groups, high-risk patients (HRG) were found to have upregulated expression levels compared with standard-risk group/medium-risk group (SRG/MRG) patients (*P* = .026, Figure S1C). Although patients with organ involvement, besides CNS, had higher *TUBB2A* expression, the difference was not statistically significant (Figure S1D).

We also performed Kaplan-Meier survival analysis, and patients with higher expression levels of *TUBB2A* (patients who are above the mean expression level) were seen to be correlated with shorter overall (OS) and relapse-free survival (RFS) rates in the pediatric T-ALL cases (*P* = .029 for OS and *P* = .042 for RFS, by log-rank test) (Figure 3A,B).

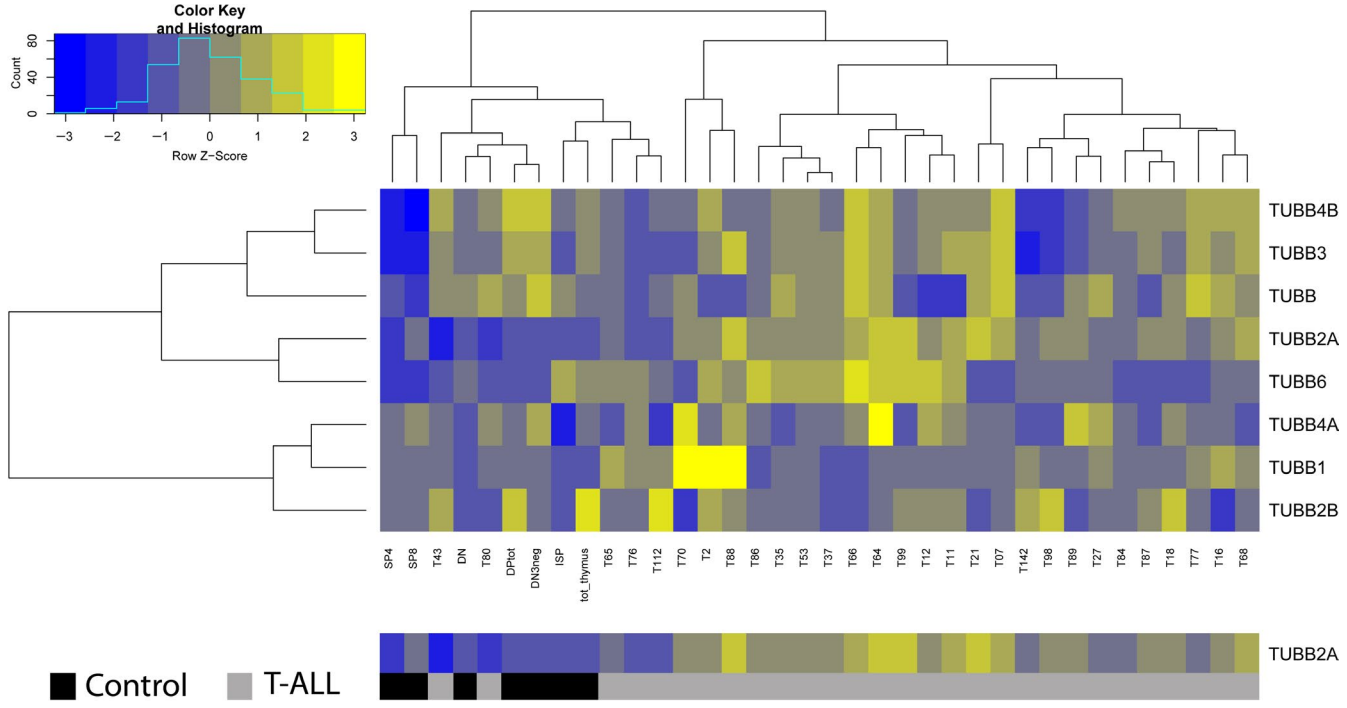


FIGURE 1 Heatmap and clustering of tubulin β isotypes in microarray data. Each row represents a transcript, and each column represents a patient. Colors were row-adjusted according to expression's *Z*-score; magenta represents downregulation, and yellow represents overexpression [Colour figure can be viewed at wileyonlinelibrary.com]

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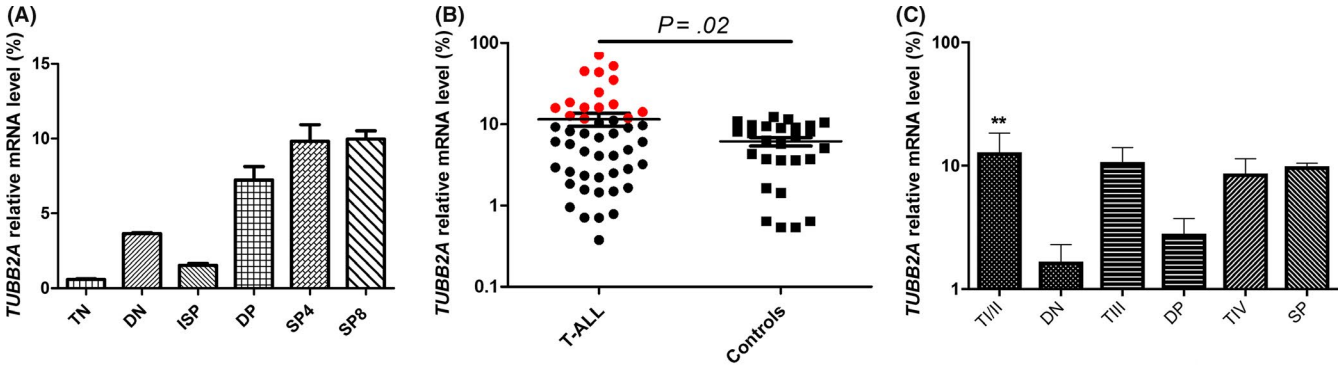


FIGURE 2 *TUBB2A*'s expression level across T cell's developmental stages and T-ALL's comparison with its respective T-cell stage. A, Expression level of *TUBB2A* across healthy T-cell subsets. TN (triple negative), DN (double negative), ISP (immature single positive), SP4 (CD4 single positive), SP8 (CD8 single positive). B, Relative *TUBB2A* expression in T-ALL and healthy control group was tested for significance with Student's *t* test. The red dots represent the samples with higher *TUBB2A* expression than the mean of all patients' levels. *Y*-axis was log10-transformed. C, *TUBB2A* expression in T-ALL phenotypes (EGIL classification) and their stage-specific healthy controls (*y*-axis was log10-transformed). ** represents $p \leq 0.01$ [Colour figure can be viewed at wileyonlinelibrary.com]

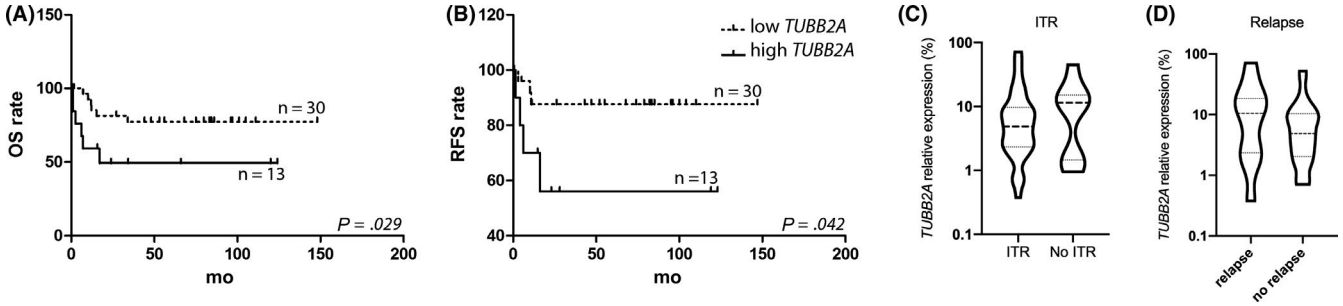


FIGURE 3 Kaplan-Meier plots of overall survival (OS) and relapse-free survival (RFS). Given *P* values were calculated with log-rank test, and indicated numbers represent patients' numbers within each group. OS (A) and RFS (B) of patients with low *TUBB2A* (dashed line) and high *TUBB2A* (solid line) expression levels (below and above mean expression level respectively). Comparison of *TUBB2A* expression levels between groups of induction therapy (ITR) response (C) and relapse (D) (*y*-axis was log10-transformed)

On the other hand, no statistical difference was found on *TUBB2A* expression levels and sex (male vs female) ($P = .35$), age (<10 years vs >10 years) ($P = .92$), induction therapy response (ITR) ($P = .61$), and relapse ($P = .27$) (Figure 3C,D). When *TUBB2A* expression, induction response, WBC, and risk group factors were used in multivariate analysis, results showed that *TUBB2A* was not significant in predicting overall survival and relapse-free survival as an independent prognostic factor.

3.4 | *TUBB2A* knockdown leading to decreased cell viability

TUBB2A's possible effect on survival of leukemic cells was tested with in vitro siRNA downregulation. We have checked *TUBB2A* mRNA expression levels in four different T-ALL cell lines (Molt4, Jurkat, Cem, and DND41) and found that the highest expression was detected in Molt4 (Figure S2A). To reverse the high *TUBB2A* expression, the Molt4 (T-ALL cell line) was transfected with *TUBB2A*-specific siRNA and knockdown effectiveness was evaluated at 24, 48, and 72 hours time points. *TUBB2A*-specific siRNA treatment suppressed *TUBB2A* mRNA levels by 55% at 24 hours ($P = .016$), 41% at 48 hours ($P = .021$), and 50% at 72 hours ($P = .043$) time points (Figure S2B). *TUBB2A*-specific siRNA treatment effects on cell viability were assessed with Annexin V-FITC and PI staining (Figure S2). When siRNA treatment was compared with mock samples, although the difference was not different at 24 hours, there were 1.14- and 1.5-fold increases in cell death at 48 and 72 hours time points, respectively (Figure S2C).

3.5 | Evaluation of Tubulin β's prognostic relevance using PRECOG

Figure S3 was adapted from database's output by excluding cancers, which contained insignificant values for all β tubulins and *TUBB5P* that had no significant *Z*-score across all cancer types (absolute *Z*-score of >1.96 corresponding to $P < .05$ was considered as being

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statistically significant). Tubulin β's clinical relevance was observed in this database, which, among other cancer types, also includes AML, B-ALL, Burkitt lymphoma, CLL, DLBCL, FL, mantle cell lymphoma, and multiple myeloma. High expression of *TUBB2A* was significantly correlated with worse survival with overall meta—*Z*-score of 2.07 across all cancers in this database.

4 | DISCUSSION

Several studies have demonstrated differing composition of tubulin isotypes in different tissue types and disease conditions.[9,12-14,20] Since microtubules are taking active part in cell division, they are among direct targets of several antimitotic drugs and are known to be correlated with diagnosis and prognosis of several cancer types.[15,21] In this study, we analyzed tubulin expression levels in expression array performed on pediatric T-ALL patients and found that the *TUBB2A* was the only tubulin isotype with significant expression changes compared with healthy thymocyte control subsets. Our results showed that the most immature T cells (TN, DN, and ISP) showed the lowest *TUBB2A* expression and the levels were increased in parallel with the maturation stages of the thymocyte development. Although Leandro-García et al[9] have found that *TUBB2A* was the major isotype of neurons and was present at lower levels in lung, kidney, spleen, stomach and thymus, there are no data on the *TUBB2A* expression levels among thymocyte subsets. On the other hand, our childhood T-ALL samples showed increased expression of *TUBB2A* and the highest expression was at the very immature stage patients compared with their developmental healthy counterpart (DN). Cunningham et al have studied the relationship between microtubule organization center (MTOC) polarization in response to TCR stimulation in immature (DP) and mature (SP) T cells.[22] Their results showed that TCR stimulation led to the polarization of microtubule-organizing center through *GSK3*'s activity (which is known to negatively regulate microtubule stability) in SP cells but not in DP cells. The authors suggested that *GSK3*'s presence in immature T cell destabilizes microtubules and inhibits immature T cells from proliferation in the presence of TCR stimulation, whereas in mature T cells, TCR stimulation in the presence of low *GSK3* activity increases T cell's survival and proliferation. These results are indicating that there is a possible impact of tubulin isotype composition on the stability of microtubules[14] in addition to microtubule-associated proteins (MAPS). Increasing manner of *TUBB2A*'s expression levels found in our study may contribute via another microtubule regulation, which in return might be altering T cells' survival and proliferation throughout development stages of thymocyte maturation. Also, during malignancy increased expression of *TUBB2A* found in our study may be conferring proliferation and survival of immature leukemic T cells. Despite the fact that developmental stage of T-ALL was shown to be useful in risk stratification of adult T-ALL,[23,24] it was shown to have no significant correlation with survival in pediatric T-ALL and only to be correlated with induction therapy.[25]

In the human ALL xenograft model, alterations of the actin and tubulin cytoskeletons were found associated with intrinsic and acquired resistance to vincristine. There is also clear evidence that specific β-tubulin isotypes differentially influence sensitivity to tubulin-binding agents (TBAs). The proportion of tubulins in the polymerized form in childhood ALL xenografts was found correlated with in vitro vincristine sensitivity.[26] TBAs are small-molecule inhibitors that have diverse structures. At high concentrations, TBAs are broadly classified as microtubule-stabilizing or microtubule-destabilizing agents according to their effects on the mass of microtubule polymers. It is now apparent that TBAs can also exert their toxic effects on cancer cells at drug concentrations that disrupt microtubule dynamics without having a marked effect on microtubule polymer levels.[27,28] High levels of polymerized tubulin were shown to have impact on microtubule stability, reduced sensitivity to microtubule-destabilizing drugs (eg, Vinca alkaloids), and increased sensitivity to microtubule polymerizing drugs (eg, paclitaxel).[29] This evidence is supported by elevated polymerized tubulin levels in vincristine-resistant human leukemia cell lines.[29,30] Berlin-Frankfurt-Münster (BFM) regimen has demonstrated improved outcomes in children with ALL,[31] and vincristine is one of the molecules used during induction therapy. Vincristine's mechanism of action is via inhibition of microtubule assembly by binding to beta-tubulin. Our pediatric ALL cohort that was treated with BFM protocol had increased *TUBB2A* expression in pediatric T-ALL patients and was found to be correlated with shorter OS and shorter RFS rates compared with normal expression levels. Recently, Martin-Guerrero et al had sequenced nine vincristine pharmacodynamics–related genes, including *TUBB2A*, in B-ALL patients and found no correlation with variations in these genes and vincristine-related neurotoxicity.[32] But the study was evaluating only one SNP region, and the *TUBB2A* expression levels were not included.

Altered expression of TUBB isotypes has been shown in a number of cancer types, which in turn may affect disease progression and response to therapy: High expression of tubulin βI in breast cancer is correlated with poor response to docetaxel[33]; low βII in breast and ovarian cancers correlates with poor response to taxane[34,35]; high βIII in non–small-cell lung cancer (NSCLC) correlates with subtype and poor survival, poor response to taxane in breast cancer, poor survival in ovarian cancer and uterine serous carcinoma, poor response to taxane in gastric cancer and advanced disease, and early recurrence in prostate cancer[36-39]; high βIVa correlates with poor response to taxol in ovarian cancer[40]; and high βV correlates with favorable response to taxane in NSCLC.[41] Alternative splicing and expression of Class II Tubulin Beta (*TUBB2B*) are also associated with outcome in diffuse large B-cell lymphoma.[42] When we checked PRECOG database to see *TUBULIN* expression effects on survival in different types of cancer samples, database showed that *TUBB2A's* expression level had negative correlation in most cancer types (Figure S2) with meta—Z-score of 2.07 (corresponding to a *P* value of <.05).[17]

TUBB2A expression was seen to be significantly higher in patients with high WBC counts (>100) at diagnosis. Host genome risk

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variants that are affecting variable WBC counts are still unknown. High WBC counts at diagnosis are known to be positively correlated with worse survival.[43-45] Children with T-ALL and high WBC (>100) at diagnosis have high risk for CNS (central nervous system) involvement.[46] Here, we also found that high-risk group patients and T-ALL cases with organ involvements had increased *TUBB2A* expression.

There are not enough data about the exact physiological functions of tubulin subtypes due to different post-translational modifications and functional complexity.[47] Previous studies have shown that high *TUBB2A* expression levels prevent lymphoblastoid cell line to undergo apoptosis under paclitaxel.[48,49] In line with this finding in our study, siRNA-mediated downregulation of *TUBB2A* led to decreased survival of Molt4 cells and showed 1.5-fold increase especially in the late apoptosis. Pro- and anti-apoptotic proteins such as *TP53* and *BCL-2* are tethered or transported by microtubules. Tubulin-binding drugs (TBDs) induce apoptosis, and cancer cells that have higher expression of pro-apoptotic proteins were found more susceptible to cell death induced by paclitaxel.[50] Our apoptosis results may have been even higher with an addition of TBD, like vincristine, and this should be evaluated in further analysis.

This study showed that *TUBB2A*, β tubulin subunit of microtubule, is elevated in pediatric T-ALL cases and its high expression is correlated with worse overall and relapse-free survival. Elevated level of *TUBB2A* seems to give a survival advantage to leukemic blasts cells in patients, which is partially supported by our in vitro findings of tubulin's siRNA-mediated suppression. Survival suppression was more evident at 72 hours time point after siRNA treatment. This finding is restricted to MOLT4 cell line, and thus, the extent of *TUBB2A* suppression's effects on other T-ALL cell lines should be validated. The knockdown on protein levels cannot be measured due to lack of *TUBB2A*-specific antibodies. All the commercially available antibodies are detecting total TUBB2 protein, and the provided information might be misleading since there is a redundancy among TUBB proteins. Microtubule-stabilizing and microtubule-destabilizing agents that are targeting tubulins are commonly used in clinics and understanding of resistance and toxicity mechanisms may lead to development of more effective drugs. Overexpression or aberrant expression of TUBB subtypes can affect the balance of the microtubules or microtubule-associated proteins, which might lead to drug resistance and relapse. Contribution of cytoskeleton proteins to drug resistance needs further investigation, and understanding the differential expression and mode of action of microtubule genes will improve better therapy strategies.

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KK, OHN, and MS designed the study. DT, TC, CT, ZK, and GNO collected the clinical information. KK, OHN, and YYN carried out experiments. KK, OHN, YE, and MS analyzed the molecular findings. KK, OZN, DT, and MS performed clinical correlations. KK, OHN, and MS wrote the draft. All authors approved the final manuscript.

CONFLICT OF INTERESTS

The authors have no competing interests.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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