Expression of Oct3/4 isoforms and its association with the risk of relapse in acute lymphoblastic leukemia

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Abstract. Oct3/4 is a transcription factor that maintains the stemness of both embryonic and adult stem cells. The Oct3/4 gene produces three isoforms: namely, Oct3/4A, Oct3/4B and Oct3/4B1. Increased Oct3/4 expression is associated with lower survival rates and chemoresistance in patients with cancer. The present study examined the association between Oct3/4 isoforms expression and the risk of relapse to acute lymphoblastic leukemia (ALL). The mRNA expression levels of Oct3/4A, Oct3/4B and Oct3/4B1 were analyzed using reverse transcription-quantitative PCR in 51 patients with ALL and 12 children without ALL. The results of the present study indicated that the mRNA expression levels of Oct3/4A (P=0.008), Oct3/4B (P=0.019) and Oct3/4B1 (P=0.025) were significantly elevated in patients with ALL. Moreover, elevated mRNA expression levels of Oct3/4A [odds ratio (OR)=13.33; 95% confidence interval (CI): 1.57-112.99; P=0.018), Oct3/4B (OR=20.00; 95% CI: 2.37-168.64; P=0.006) and Oct3/4B1 (OR=7.26, 95% CI: 1.43-36.93; P=0.017) isoforms were associated with a heightened risk of relapse in patients with ALL. The multivariate analysis, stratified using established prognostic factors, identified Oct3/4A, Oct3/4B and Oct3/4B1 mRNA expression levels as independent prognostic markers of ALL. The results of the present study also indicated that the expression levels of Oct3/4A (P=0.0112), Oct3/4B (P=0.0044) and Oct3/4B1 (P=0.0071) were significantly elevated in $CD34^+/CD38^-$ patients with ALL compared with those in $CD34^+/CD38^+$ patients with ALL. Thus, increased mRNA expression levels of Oct3/4A, Oct3/4B and Oct3/4B1 may be associated with a poor prognosis in patients with ALL.

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Introduction

Acute lymphoblastic leukemia (ALL) is characterized by the uncontrolled proliferation of lymphoid precursor cells. The ALL-B subtype constitutes a substantial portion of leukemia cases in the pediatric population, accounting for ~80% of diagnoses. By contrast, the T-lymphoid subtype accounts for 10-15% of childhood ALL cases (1).

The prognosis of leukemia depends on the type and characteristics of the patient at diagnosis, including cytogenetic and molecular alterations, the count of white blood cells at diagnosis, response to primary therapy, and the phenotype of the blasts (B and T lymphoid precursor cells and myeloid precursor cells) (2). Current treatments have led to significant increases in patient survival rates, with improvements of up to 90% in five years. However, a percentage of patients do not respond and relapse (3). This event is associated with cancer stem cells (CSCs), which play a crucial role in resistance to chemotherapy and radiotherapy, leading to chemo-radioresistant tumors (4).

The majority of genes associated with hematological malignancies code for transcription factors, and these play key roles in regulating the cell cycle, and in the proliferation, differentiation and survival of lymphoid and myeloid precursors (5).

Notably, CSCs participate in the origin and progression of cancers (6).

Oct3/4 (also known as Pou5f1, Oct3, OTF3 and OTF4) is a transcription factor located in stem cells. Its expression is associated with pluripotency and self-renewal, making it a key marker for CSCs. The Oct3/4 gene generates three isoforms via alternative splicing, each with different functions (7). For example, Oct3/4A is located in the nucleus of embryonic stem cells (ESCs) and CSCs, and functions as a transcription factor to maintain cell pluripotency; Oct3/4B is located in the cytoplasm of cancer cells, and cannot sustain the pluripotency of ESCs (8) and exhibits anti-apoptotic functions (9). Oct3/4B1 is located in both the cytoplasm and nucleus of pluripotent cells (10), and participates as an anti-apoptotic factor in tumorigenesis (11).

Results of previous studies revealed that Oct3/4 is a prognostic marker of disease, and elevated expression of Oct3/4 has been reported in solid tumors (12-15). In individuals with acute myeloid leukemia (AML), Oct3/4 expression is elevated in leukemic stem cells, specifically in $CD34^+$ $CD38^-$ cells (16). In addition, Oct3/4 has been recognized as a negative prognostic factor associated with lower survival rates. Results of previous studies also indicated that elevated Oct3/4 expression levels may act as a prognostic marker in patients with leukemia (17-19). However, the expression levels of Oct3/4A, Oct3/4B and Oct3/4B1, and the specific association with the risk of relapse in patients with ALL, are yet to be fully elucidated. Thus, the present study aimed to analyze the expression of Oct4A, Oct4B and Oct4B1, and determine whether the expression of Oct3/4 isoforms is associated with relapse in patients with ALL.

Materials and methods

Data source and Gene Expression Omnibus (GEO) data sets. GSE7638 (20), GSE635 (21), GSE9006 (22) and GSE5820 (23) microarray datasets were downloaded from the GEO (http://www.ncbi.nlm.nih.gov/geo) and Gene Expression database of Normal and Tumor tissues 2 (GENT2; http://gent2.appex.kr/gent2/) databases. The platform, including four microarrays, was GPL96 [(HG-U133A) Affymetrix Human Genome U133A Array]. Data for non-malignant ALL, based on morphological criteria, cytochemical staining, and immunophenotyping of blast cells (reported by hospitals, including St. Jude Children's Research Hospital and the Hematology/Oncology Department of Children's Hospital), were used to assess potential differences in Oct3/4 expression.

Study population. Bone marrow (patients with ALL) and blood (healthy individuals) samples were collected in the Pediatric Oncology Service of the State Cancer Institute (SCI) from the South of Mexico (Acapulco, Mexico), between September 2016 and April 2020. The samples were placed in tubes with an anticoagulant (EDTA) and stored in the biobank of the laboratory of Molecular Biomedicine at the FCQB of Autonomous University of Guerrero. Leukocytes were purified by selective osmotic lysis, as previously described by Gómez-Gómez *et al* (24). Total RNA was isolated from the bone marrow and blood samples using the TRIzol® method (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Total RNA was stored at -80˚C in the biobank of the laboratory of Molecular Biomedicine at the FCQB of Autonomous University of Guerrero.

The present study involved case and control groups: Cases included samples (total RNA) of patients diagnosed with ALL (n=51), based on established clinical, cytomorphological, molecular and immunological criteria. The study also involved 12 healthy individuals as the control group ($4\text{-}10\text{x}10^3$ leukocytes/mm^3) in the absence of a family history of leukemia. In 2020, RNA samples were received from the biobank of the laboratory of Molecular Biomedicine at the FCQB of Autonomous University of Guerrero (Chilpancingo, Mexico). The bone marrow and blood samples were obtained from patients as part of the samples taken for clinical diagnostic tests. The present study was approved by the ethics committee of the Institute of Cancer of the State of Guerrero, Mexico (approval no. FO-INV-AUT-2018) and by the committee of the biobank of the laboratory of Molecular Biomedicine at the FCQB of Autonomous University of Guerrero (Chilpancingo, Mexico) (approval no. BB-LBM-03-2020). All participants or their guardians provided written informed consent following a thorough explanation of the study's objectives Relapse is characterized by the emergence of blast cells in the marrow or the presence of localized leukemic infiltrates at any site following the conclusion of induction chemotherapy, according to protocols previously established (24,25).

Reverse-transcription quantitative (RT-q) PCR. A total of 500 ng of RNA was reverse-transcribed into cDNA using the SuperScript II First-Strand Synthesis System (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. qPCR was performed using the following thermocycling conditions: 65˚C for 10 min, 22˚C for 10 min, 42˚C for 90 min, and 75˚C for 5 min. cDNA was stored at -20˚C for subsequent experiments. qPCR was performed using a total volume of 15 μl, consisting of 7.5 μl of 2X TaqMan Universal PCR Master Mix II, 0.5 μM of each primer, 200 ng of template per reaction, and ultrapure water. Primer sequences and product sizes are outlined in Table SI, and the efficiency and specificity of these primers were plotted by Asadi *et al* (26) and are outlined in Table SI. mRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (27). and normalized to the internal reference gene, GAPDH.

Statistical analysis. Continuous data are presented as the mean ± standard deviation (SD) and/or median with the 25th and 75th percentiles. Categorical data comparisons were conducted using Chi-squared or Fisher's exact tests. The Mann-Whitney test was employed to assess differences in the expression levels of Oct3/4A, Oct3/4B and Oct3/4B1 between groups. The association between Oct3/4A, Oct3/4B and Oct3/4B1 expression and the risk of relapse in patients with ALL was evaluated using odds ratios (ORs) and 95% confidence intervals (CIs) derived from univariate and multivariate logistic regression analyses. Statistical analysis was performed using SPSS (version 20.0; IBM Corp.) and GraphPad Prism (version 5.0; GraphPad Software, Inc.; Dotmatics). $P<0.05$ was considered to indicate a statistically significant difference.

Table I. General characteristics and clinicals of the individuals.

Clinicopathological characteristics	ALL (n=51)	Controls (n=12)
Age, years (mean + SD)	8.321+3.99	10.58+7.20
Leukocyte count, /mm^3	16,300 (6,700-37,500)[a]	6,700 (6,250-8,450)[a]
Sex, n (%)		
Female	26 (50.98)	7 (58.33)
Male	25 (49.02)	5 (41.67)
Status of individuals		
Alive	31 (60.78)	12 (100.00)
Decreased	20 (39.22)	-
Relapse		
No	21 (41.18)	-
Yes	30 (58.82)	-
Risk by age and leukocytes at diagnosis		
Low-risk	26 (50.98)	-
High-risk	25 (49.02)	-
Inmunophenotype		
B-lineage	51 (100.00)	-
Chromosomal translocation		
BCR-ABL [t(9;22)]	1 (1.96)	-
ETV6-RUNX1 [t(12;21)]	4 (7.84)	-
del1(p32) (STIL-TAL1)	2 (3.92)	-
None	44 (86.28)	-
CD34 expression		
CD34$^-$	24 (47.06)	-
CD34$^+$	22 (43.14)	-
no data	5 (9.80)	-
CD34/CD38 expression		
CD34$^+$CD3^8+	10 (45.45)	-
CD34$^+$CD38$^-$	12 (54.55)	-

Data are expressed as n (%), unless otherwise indicated. [a]Median (percentiles 25-75). ALL, acute lymphoblastic leukemia; low-risk group, aged between 1-10 years with <50,000 leukocytes/mm^3; high-risk group, aged <1 and >10 years with >50,000 leukocytes/mm^3.

Results

Oct3/4 is expressed in patients with ALL obtained from the GEO data sets. Analysis of mRNA expression levels in patients with ALL and control samples obtained from the GSE datasets in GEO and GENT2 databases indicated that Oct3/4 expression in patients with ALL was higher than in healthy individuals [Fig. 1A (GSE9006/GSE5820) and Fig. 1B (GSE7638/GSE635)]. To the best of our knowledge, there are no previous literature describing the presence of the Oct3/4 isoforms in patients with ALL.

Population characteristics. mRNA samples of patients with ALL were obtained from the biobank of the laboratory of Molecular Biomedicine at the FCQB of Autonomous University of Guerrero. The present included participants with a mean age of 8.32±3.99 years, and the median leukocyte count at diagnosis was 16,300 leukocytes/mm^3. Among patients with ALL, 49.02% were categorized as high-risk, and 58.82% experienced a relapse during treatment. By contrast, healthy controls exhibited a mean age of 10.58±7.20 years, with a normal leukocyte count ranging from 4 to 10x10^3 leukocytes/mm^3, and a median of 6,700 leukocytes/mm^3. The characteristics of all participants are presented in Table I.

Oct3/4A, Oct3/4B and Oct3/4B1 are expressed at high levels in patients with ALL. To determine whether the expression levels of Oct3/4 isoforms differ between patients with ALL and healthy individuals, samples from 51 patients with ALL and 12 healthy controls, the expression of Oct3/4A, Oct3/4B and Oct3/4B1 was analyzed in samples from 51 patients with ALL and 12 healthy controls. The results of the present study indicated a significant increase in Oct3/4 isoforms expression in patients with ALL (Fig. 2). In patients with ALL, the expression levels of Oct3/4A (median, 8.55; P=0.008; Fig. 2A and Table II), Oct3/4B (median, 6.62; P=0.019; Fig. 2B and Table II) and Oct3/4B1 (median, 14.42; P=0.025; Fig. 2C and

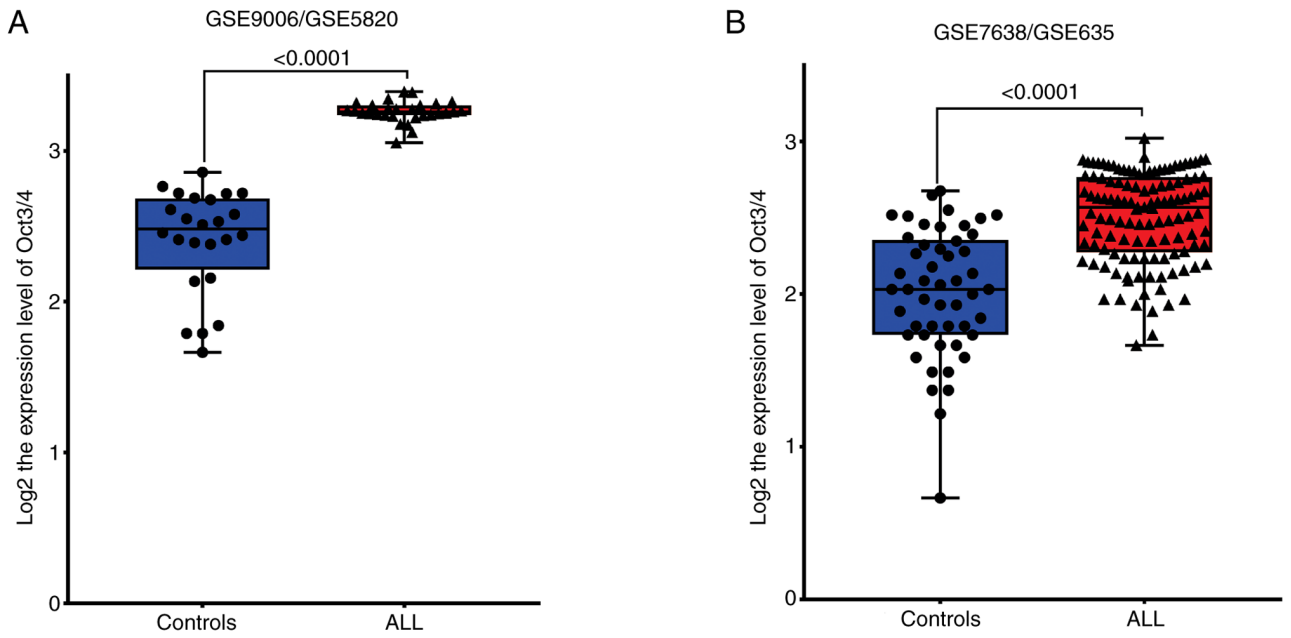

Figure 1. Oct3/4 expression is increased in ALL. (A) and (B) Analysis of the cBioPortal database indicated that Oct3/4 levels were elevated in patients with ALL. The boxplot represents log2 values on a logarithmic scale. Results obtained from the GEPIA database confirmed that Oct3/4 expression was elevated in patients with ALL. All samples used in this analysis were sourced from the Cancer Genome Atlas dataset. ALL, acute lymphoblastic leukemia; GEPIA, Gene Expression Profiling Interactive Analysis.

Table II. Oct3/4 isoforms mRNA expression in childhood ALL and healthy individuals.

		Mean	Std. deviation	Std. error of the mean	95% CI of mean	Median	25-75% percentiles	P-value
Oct3/4A	Controls	1.67	1.94	0.56	0.44-2.90	0.52	0.11-3.27	0.008
	ALL	45.42	118.50	16.60	12.08-78.75	8.55	0.32-46.86	
Oct3/4B	Controls	2.44	2.97	0.86	0.56-4.33	0.61	0.18-4.70	0.019
	ALL	28.02	50.59	7.08	13.79-42.25	6.62	0.52-40.83	
Oct3/4B1	Controls	3.22	3.06	0.88	1.27-5.16	1.97	0.33-6.73	0.025
	ALL	60.99	107.80	15.09	30.68-91.31	14.42	0.71-81.09	

Obtained by Mann Whitney test. OR, odds ratio; 95% CI, confidence interval; ALL, acute lymphoblastic leukemia.

Table II) were significantly elevated, compared with those in healthy individuals.

Oct3/4 isoforms expression is associated with relapse in patients with ALL. Patients with ALL were categorized into two groups: namely, those with relapse (n=30) and those without relapse (n=21). The results of the present study indicated that the expression levels of Oct3/4A (P=0.0004; Fig. 3A and Table III), Oct3/4B (P=0.0004; Fig. 3B and Table III), and Oct3/4B1 (P=0.0005; Fig. 3C and Table III) were significantly higher in patients with ALL with relapse compared with those in patients without relapse. To assess the association between Oct3/4 isoforms expression and the risk of ALL relapse, a logistic regression analysis was performed. Patients were categorized into two groups based on their expression levels (low- and high-expression). The mean expression level of Oct3/4A (mean, 45.42; Fig. 3A and Table II), Oct3/4B (mean, 28.02; Fig. 3B and Table II), and Oct3/4B1 (mean, 60.99; Fig. 3C and Table II) was used as the cut-off point to divide all 51 patients with ALL into two groups (Table II). Those who expressed Oct3/4 isoforms at levels less than the cut-off value were assigned to the low expression group [Oct3/4A (n=38); Oct3/4B (n=35); Oct3/4B1 (n=36)], and those with expression levels above the cut-off value were assigned to the high expression group [Oct3/4A (n=13); Oct3/4B (n=16); Oct3/4B1 (n=15)].

The results of the present study revealed an association between the expression of Oct3/4 isoforms (Oct3/4A, Oct3/4B and Oct3/4B1) and relapse risk in patients with ALL (P<0.05). Patients with ALL exhibiting high expression levels of Oct3/4 isoforms exhibited a notable increase in relapse risk: Oct3/4A (OR=13.33; 95% CI: 1.57-112.99; P=0.018), Oct3/4B (OR=20.00; 95% CI: 2.37-168.64; P=0.006) and Oct3/4B1 (OR=7.26; 95% CI: 1.43-36.93; P=0.017).

Age, leukocyte count at diagnosis, and the expression of Oct3/4A, Oct3/4B and Oct3/4B1 were included in a multivariate analysis to assess whether the aforementioned Oct3/4

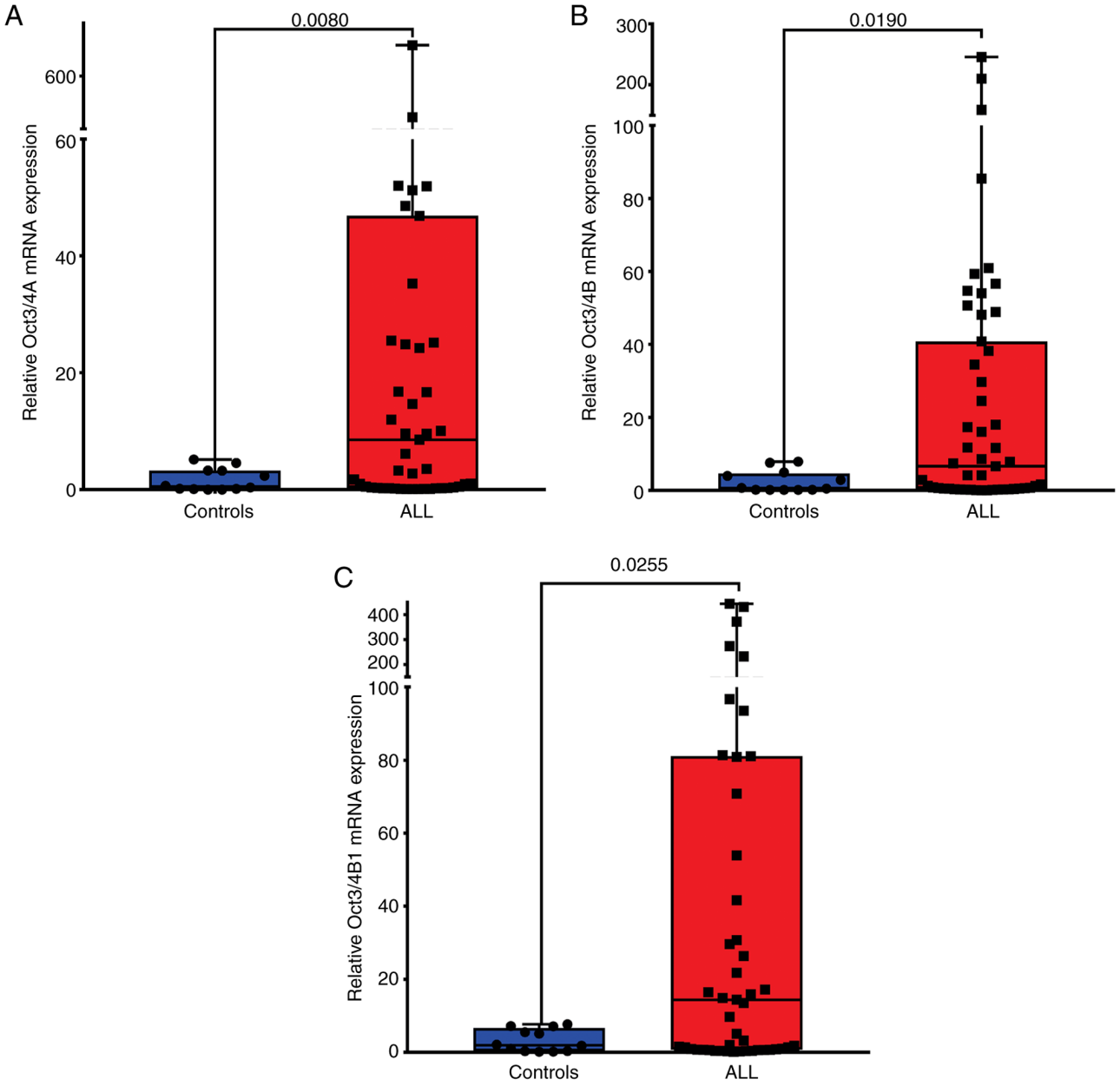

Figure 2. Expression patterns of Oct3/4A, Oct3/4B and Oct3/4B1 in patients with ALL. The mRNA expression levels of Oct3/4 isoforms were significantly higher in patients with ALL compared with healthy individuals. (A) Oct3/4A, median (25-75th percentiles) at 8.55 (0.32-46.86; P=0.008). (B) Oct3/4B, 6.62 (0.52-40.83; P=0.019). C) Oct3/4B1, 14.42 (0.71-81.09; P=0.025). ALL, acute lymphoblastic leukemia.

isoforms are independent predictors of relapse risk. The results of the present study revealed that Oct3/4A (OR=18.24; 95% CI: 2.03-164.29; P=0.010), Oct3/4B (OR=42.86; 95% CI: 4.25-431.31; P=0.001) and Oct3/4B1 (OR=14.08; 95% CI: 2.31-85.67; P=0.004) expression levels serve as independent prognostic indicators for patients with ALL (Table IV). Collectively, these results suggested that the expression of these Oct3/4 isoforms may play a role in the relapse of patients with ALL.

Overall survival based on the expression levels of Oct3/4 isoforms. The association between the expression levels of Oct3/4 isoforms and the overall survival of patients with ALL was investigated in the present study. In patients with ALL, Kaplan-Meier analysis revealed no significant difference in overall survival or the expression levels of Oct3/4 isoforms (log-rank test; P>0.05; Fig. 4).

Expression levels of Oct3/4A, Oct3/4B and Oct3/4B1 are high in CD34⁺ patients with ALL. Surface marker CD34 is used to identify and isolate hematopoietic stem/progenitor cells (HSC/HPC) (28). $CD34^+$ B-ALL cells are used in the diagnosis and prognosis of patients with ALL (29). To determine whether Oct3/4 isoforms are expressed differently between $CD34^+$- and $CD34^-$- patients with ALL, samples from 51 patients with ALL that were positive for Oct3/4A, Oct3/4B and Oct3/4B1 were analyzed. The results of the present study indicated a significant increase in Oct3/4 isoforms expression in $CD34^+$-patients with ALL [Oct3/4A (median, 13.41; P=0.0368; Fig. 5A and Table SII); Oct3/4B (median, 9.76;

Table III. Oct3/4 isoforms mRNA expression in childhood AL with and without relapse.

		Mean	Std. deviation	Std. error of the mean	95% CI of the mean	Median	25-75% percentiles	P-value
Oct3/4A	Without relapse	6.90	15.71	3.43	-0.24-14.05	0.38	0.22-7.35	0.0004
	With relapse	72.38	149.10	27.22	16.70-128.00	25.02	3.49-716.60	
Oct3/4B	Without relapse	6.96	14.47	3.16	0.37-13.55	0.53	0.24-9.47	0.0004
	With relapse	42.76	61.02	11.14	19.98-65.55	20.72	3.52-245.80	
Oct3/4B1	Without relapse	14.34	32.59	7.11	-0.50-29.18	0.94	0.52-9.76	0.0005
	With relapse	93.65	128.90	23.54	45.51-141.80	35.64	8.12-121.40	

Obtained by Mann Whitney test. OR, odds ratio; 95% CI, confidence interval.

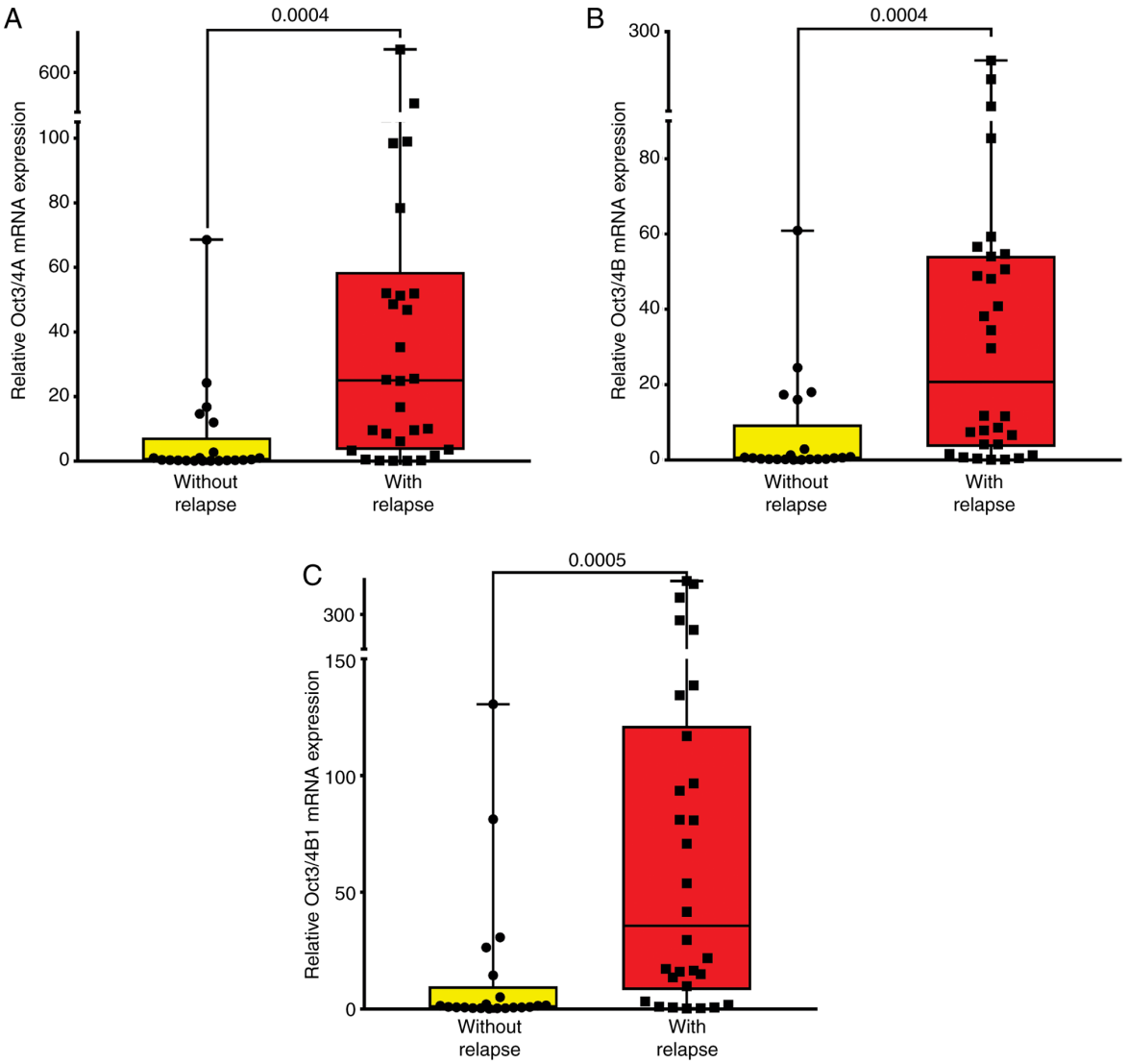

Figure 3. Expression patterns of Oct3/4A, Oct3/4B and Oct3/4B1 in patients with ALL, both with and without relapse. mRNA expression levels of Oct3/4 isoforms were significantly elevated in patients with ALL experiencing relapse. Results are displayed as the median (25-75th percentiles). (A) Oct3/4A, 25.02 (3.49-716.60; P=0.0004). (B) Oct3/4B, 20.72 (3.52-245.80; P=0.0004). (C) Oct3/4B1, 35.64 (8.12-121.40; P=0.0005). ALL, acute lymphoblastic leukemia.

Table IV. Association of Oct3/4 isoforms mRNA expression with the risk of relapse to ALL.

	Without relapse	With relapse	P-value[a]	Unadjusted[b]			Adjusted[c]		
				OR	CI 95%	P-value	OR	CI 95%	P-value
Risk by age									
Low-risk	15 (71.43)	16 (53.33)	0.250	1.00					
High-risk	6 (28.57)	14 (46.67)		1.09	0.94-1.26	0.239			
Risk by leukocytes at diagnosis									
Low-risk	18 (85.71)	22 (73.33)	0.490	1.00					
High-risk	3 (14.29)	8 (26.67)		2.18	0.50-9.45	0.297			
Oct3/4A									
Low-levels	20 (95.24)	18 (60.00)	0.007	1.00					
High-levels	1 (4.76)	12 (40.00)		13.33	1.57-112.99	0.018	18.24	2.03-164.29	0.010
Oct3/4B									
Low-levels	20 (95.24)	15 (50.00)	0.001	1.00					
High-levels	1 (4.76)	15 (50.00)		20.00	2.37-168.64	0.006	42.86	4.25-431.31	0.001
Oct3/4B1									
Low-levels	19 (90.48)	17 (56.67)	0.012	1.00					
High-levels	2 (9.52)	13 (43.33)		7.26	1.43-36.93	0.017	14.08	2.31-85.67	0.004

Data are expressed as n (%) unless indicated otherwise. Risk of relapse to ALL [values were calculated taken as reference groups to 1-10 years (Low-risk), <50,000 leukocytes/mm^3 (low-risk), Low-levels (Oct3/4A, Oct3/4B and Oct3/4B1)]. [a]P-value was obtained by the Fisher's exact test. [b]OR non-adjusted. [c]OR adjusted by age at diagnosis (low-risk: 1-10 years and high-risk: <1 and >10 years), leukocytes at diagnosis (low-risk: <50,000 leukocytes/mm^3 and high-risk: >50,000 leukocytes/mm^3), and Oct3/4 isoforms mRNA expression levels (low-levels and high-levels). OR, odds ratio; CI, confidence interval; ALL, acute lymphoblastic leukemia.

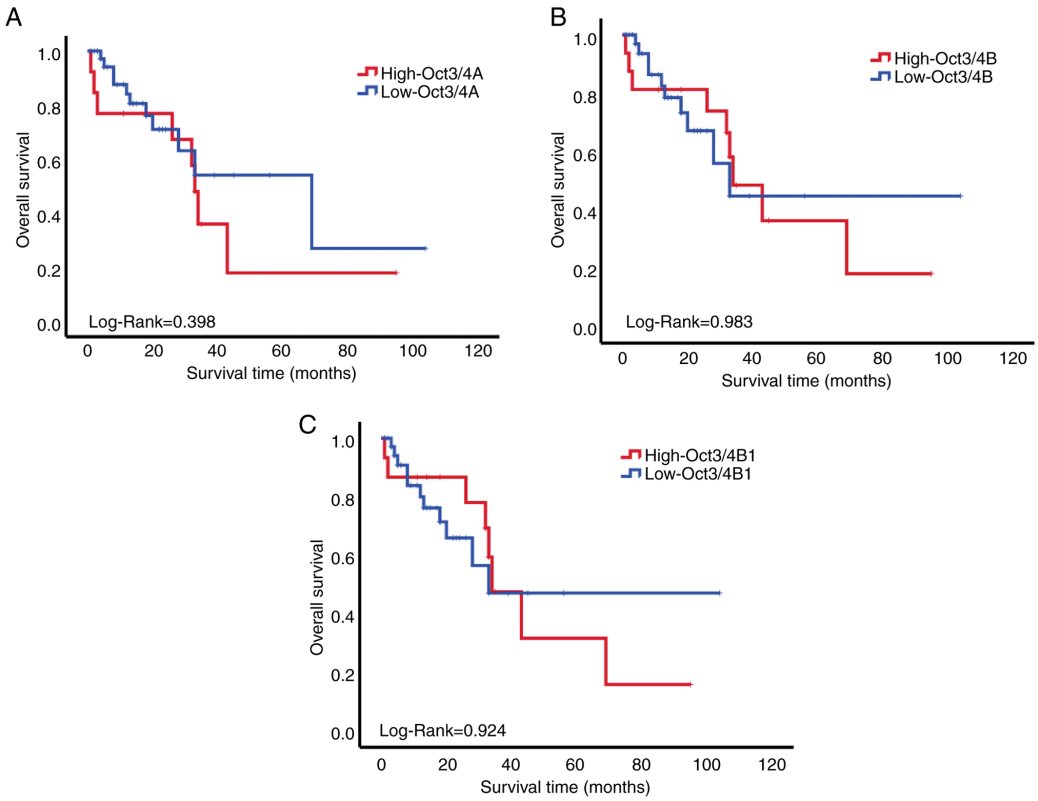

Figure 4. Kaplan-Meier curves were used to demonstrate the potential association between mRNA expression levels of Oct3/4 isoforms and the OS of patients with ALL. (A) OS in patients with ALL with high or low Oct3/4A expression (P=0.398). (B) OS in patients with ALL with high or low Oct3/4B expression (P=0.983). (C) OS in patients with ALL with high or low Oct3/4B1 expression (P=0.924). OS, overall survival; ALL, acute lymphoblastic leukemia.

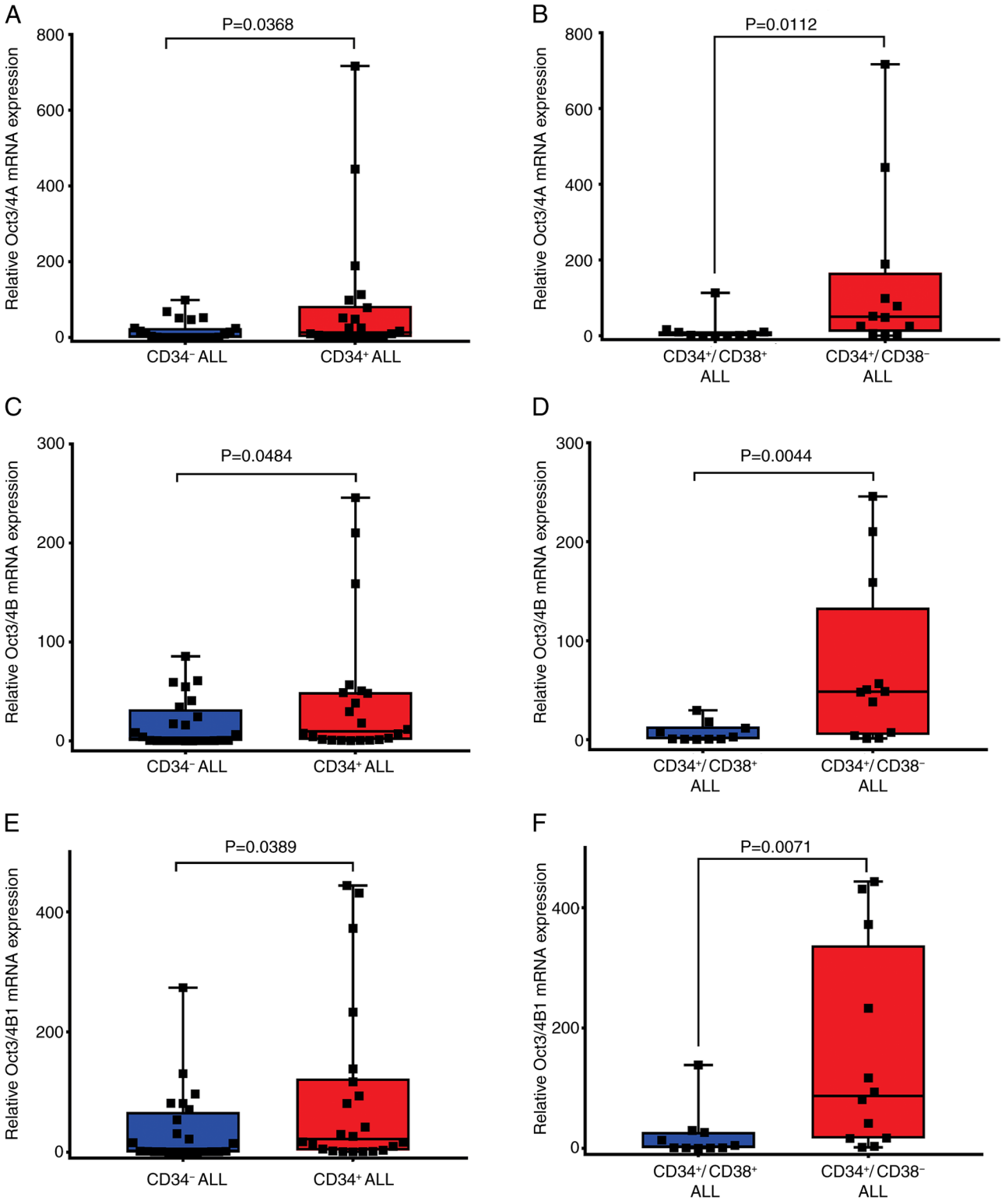

Figure 5. Expression patterns of Oct3/4A, Oct3/4B and Oct3/4B1 in $CD34^+$ and $CD34^-$ patients with ALL. The mRNA expression levels of Oct3/4 isoforms were significantly elevated in $CD34^+$ patients with ALL ($P<0.05$). Results are displayed as the median (25-75th percentiles). (A) Oct3/4A, 13.41 (1.55-83.40; P=0.0368). (C) Oct3/4B, 9.76 (1.18-245.80; P=0.0484). (E) Oct3/4B1, 21.79 (2.89-122.40; P=0.0389). In $CD34^+/CD38^-$ patients with ALL, the expression levels of Oct3/4 isoforms were significantly elevated ($P<0.05$). (B) Oct3/4A, 50.25 (10.90-166.50; P=0.0112). (D) Oct3/4B, 48.49 (4.97-133.40; P=0.0044). (F) Oct3/4B1, 87.33 (16.64-337.50; P=0.071). Statistical significance was determined at $P<0.05$. A Mann-Whitney test was utilized to evaluate the differences in mRNA levels. ALL, acute lymphoblastic leukemia.

P=0.0484; Fig. 5C and Table SII); Oct3/4B1 (median, 21.79; P=0.0389; Fig. 5E and Table SII). $CD34^+$ patients with ALL were subdivided into two groups: namely, $CD34^+/CD38^+$ (n=10) and $CD34^+/CD38^-$ (n=12), based on the expression of the CD34 and CD38 cell antigens in leukemia cells. In $CD34^+/CD38^-$ patients with ALL, the expression levels of Oct3/4A (median, 50.25; P=0.0112; Fig. 5B and Table SII), Oct3/4B (median, 48.49; P=0.0044; Fig. 5D and Table SII) and

Oct3/4B1 (median, 87.33; P=0.071; Fig. 5F and Table SII) were significantly elevated compared with those in $CD34^+/CD38^+$ patients with ALL.

Discussion

ALL is characterized by the uncontrolled proliferation of blast cells, representing 80% of pediatric ALL cases (1). This population of blasts is sustained by rare leukemia-inducing cells known as leukemic stem cells (LSCs) (30,31). LSCs ($CD34^+CD38^-$ cells) represent an immature leukemic compartment characterized by the expression of the Oct3/4 protein (16). The human Pou5f1 (Oct3/4) gene produces at least three transcripts: Oct3/4A, Oct3/4B and Oct3/4B1; and four protein isoforms: Oct3/4A, Oct3/4B-190, Oct3/4B-265 and Oct3/4B-164, through alternative splicing and initiation of translation (32). Oct3/4A is located in the nucleus and acts as a transcription factor, while OCT4B primarily resides in the cytoplasm, providing cells with resistance to apoptotic death as well as stress from heat shock or genotoxic factors (33,34). Moreover, Oct3/4B1 regulates and maintains the undifferentiated state of stem cells (35). The present study aimed to investigate the mRNA expression levels of Oct3/4A, Oct3/4B and Oct3/4B1, and determine the association between expression and relapse risk in ALL. Oct3/4 acts as a transcription factor, playing a role in tumorigenesis and metastasis. Notably, this transcription factor may be associated with unfavorable outcomes for patients with cancer (36). Previous studies have outlined the role of Oct3/4 in various tumors. However, research on the expression, characteristics and functions of Oct3/4 isoforms in children with ALL remains limited.

The results of the present study demonstrated that Oct3/4 isoforms were expressed at higher levels in patients with ALL compared with those without ALL (P<0.05). These findings are comparable with those of previous studies that also reported elevated Oct3/4 expression in patients with AML (17,18) and cervical cancer (37,38). Zhao *et al* (39) studied Oct3/4 mRNA expression in individuals with acute and chronic leukemia and revealed that expression was predominantly higher in patients with acute leukemia compared with those with chronic leukemia and the control group. By contrast, Yin *et al* (18) revealed that the expression of Oct3/4 was significantly higher in patients with AML compared with controls (18). Picot *et al* (16) and Xiang *et al* (19) reported significantly elevated levels of Oct3/4 mRNA in leukemic cell lines and patients with AML compared with their respective controls (16,19). Collectively, these findings suggested that increased expression of Oct3/4 may promote the proliferation and survival of leukemic cells. Moreover, Oct3/4 may play a significant role in the pathogenesis of leukemia and exhibits potential as a molecular target for novel treatment strategies (39).

Oct3/4A is the primary isoform associated with stemness in cancer cells. The results of the present study demonstrated that Oct3/4A and Oct3/4B mRNA expression levels were elevated in children diagnosed with ALL, compared with those without ALL. In addition, Oct3/4B may play a role in the cellular stress response (32). Li *et al* (9) revealed that the Oct3/4B isoforms enhances angiogenesis in cervical cancer through the upregulation of vascular endothelial growth factor, highlighting the oncogenic role of OCT4B (9). A previous study demonstrated that hypoxia activates OCT4B through a HIF2α-dependent pathway in lung cancer cells, promoting epithelial-mesenchymal transition, ultimately leading to cell invasion, migration and metastasis (40).

Oct3/4B1 plays a role in both pluripotency and tumorigenesis, through inhibition of apoptosis and dysregulation of the cell cycle (22). The results of the present study revealed that OCT4B1 mRNA expression was significantly higher in children with ALL, compared with those without ALL. Notably, the findings of the present study are comparable with those of previous studies, which highlighted the positive regulation of OCT4B1 transcription across various cancers, including gastric, colorectal and bladder cancer. A previous study revealed that OCT4B1 inhibits apoptosis in gastric cancer, highlighting its crucial role in tumor initiation and progression (41).

To assess the clinical significance of Oct3/4 isoforms expression in ALL, logistic regression analysis was conducted to explore the potential association between the clinical characteristics of patients with ALL and their relapse risk. The results of the present study revealed that patients who experienced relapse exhibited a higher mRNA expression of Oct3/4 isoforms than those who did not experience relapse.

The results of the present study also demonstrated a notable association between Oct3/4 isoforms expression levels and the risk of leukemia relapse. Similarly, Aref *et al* (42) reported that adults with acute leukemia who experienced relapse exhibited significantly elevated Oct3/4 levels compared with those in remission (42). Further previous studies revealed that elevated levels Oct3/4 mRNA or protein expression levels were associated with unfavorable clinical outcomes and chemoresistance across various cancers, including bladder cancer (43), ovarian (44), osteosarcoma (45), liver (46), melanoma (47), prostate, rectal, glioma, medulloblastoma, hepatocellular carcinoma and esophageal squamous cell carcinoma (4). High levels of Oct3/4 are proposed as crucial oncogenic factors in cancer development, significantly contributing to tumorigenesis, metastasis and invasion, while also suppressing apoptosis through the activation of various signaling pathways, such as WNT/β-catenin and PI3K/Akt pathways (48,49). Collectively, these results indicated that the expression levels of Oct3/4 isoforms could play a crucial role in ALL.

Oct3/4 isoforms were not significantly correlated with overall survival; however, it was observed that patients with high expression of Oct3/4 isoforms had a worse survival rate, consistent with other studies (19,42,50). However, results of the multivariate analysis revealed that patients with high expression of Oct3/4 isoforms exhibited significant OR estimates. This significance persisted alongside other prognostic factors, including age, sex and leukocyte count at diagnosis, indicating that Oct3/4 isoforms expression is an independent prognostic marker for ALL.

In addition, the results of previous studies revealed that $CD34^+$ cells are characterized by the expression of the Oc3/4 protein (16,17). The results of the present study revealed that levels of Oct3/4A, Oct3/4B and Oct3/4B1 were significantly elevated in $CD34^+$ patients with ALL, compared with those in $CD34^-$ patients with ALL. The results of the present study are comparable with those observed by Gaafar *et al* (51), who reported that the HSC/HPC subsets expressed pluripotency or stemness genes (SOX2, Nanog and OCT4). Additionally, it has been previously reported that $CD34^+$ B-ALL cells can be used in the diagnosis and prognosis of patients with ALL (25). A

recent study also demonstrated that $CD34^+/CD38^-$ leukemia cells are associated with a poor prognosis in patients with ALL (52).

Notably, Oct3/4 may play a role in relapse and therapy response in patients with ALL via numerous mechanisms: (i) Oct3/4 may directly promote the expression of miR-125b, which inhibits its direct target BCL2 Antagonist/Killer 1, resulting in the reduced apoptosis of cancer cells (37); (ii) Oct3/4 may modulate the survivin/STAT3 signaling pathway (53), or this transcription factor may enhance; and (iii) Oct3/4 enhances ATP-binding cassette protein activity in chemotherapy-resistant cancer cells (54).

The present study exhibits limitations. For example, the sample size was small, comprising only 51 patients, and more investigations are required to verify the obtained results. Furthermore, additional investigations involving larger sample sizes are necessary. Collectively, the results of the present study indicated that Oct3/4 isoforms may be associated with a poor prognosis in children with ALL. To the best of our knowledge, the present study is the first to demonstrate that Oct3/4 isoforms may act as independent markers for predicting clinical outcomes in these patients. In addition, the results highlighted the potential role of Oct3/4 isoforms expression in the risk and relapse of childhood ALL; however, future studies are needed to explain the underlying molecular mechanism of the Oct3/4 isoforms in ALL and to reveal new perspectives on the potential role of the Oct3/4 isoforms regulation in patients with ALL. In conclusion, Oct3/4 may exhibit potential as a therapeutic target, particularly in childhood ALL.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

JON and YGG developed methodology. YGG and MALV conceptualized the study. JON, YGG, CYGS, EGSB, MIZG, ABRR, MVSH, OAP, FITR, LCAR and BIA conducted investigation. ABRR, MVSH, OAP, BIA and MALV provided resources. JON, YGG, CYGS and BIA validated data. JON, YGG, CYGS and EGSB conducted formal analysis. YGG and MALV supervised the study, performed project administration, acquired funding, and wrote, reviewed and edited the manuscript. JON wrote the original draft. JON, YGG, CYGS and MALV confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the ethics committee of the Institute of Cancer of the State of Guerrero, Mexico (approval no. FO-INV-AUT-2018) and by the committee of the biobank of the laboratory of Molecular Biomedicine at the FCQB of Autonomous University of Guerrero (Chilpancingo, Mexico) (approval no. BB-LBM-03-2020). All participants or their guardians provided written informed consent following a thorough explanation of the study's objectives.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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