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DNA Methylation and the Expression of the Tumor-Suppressor Genes Protocadherin-10 and Reprimo in Pediatric Acute Lymphoblastic Leukemia

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Abstract

Background: Acute lymphoblastic leukemia (ALL) is the most prevalent hematologic malignancy among children. DNA methylation is well known to play a role in the initiation and progression of cancer. This research aimed to characterize the epigenetic inactivation and gene expression of Protocadherin-10 (PCDH10) and Reprimo (RPRM) in pediatric acute lymphoblastic leukemia, which is associated with epigenetic silencing in human cancers.

Materials and Methods: This case-control study included 21 children (mean age, 4.5 years) with new cases of ALL. In addition, 15 patients with ALL were selected following treatment. Samples of bone marrow were extracted from each patient. Prior to and following treatment, 15 paired samples were analyzed. In addition, ALL patients were compared to 18 normal controls in a case study. MS-HRM and quantitative real-time PCR were utilized to examine the DNA-promoter methylation and gene expression of two TSGs from each group.

Results: Expression of PCDH10 was significantly up regulated in treatment ALL group compared to the new cases of ALL patients (P= 0.0119), PCDH10 gene expression was higher in the normal control group than in the new cases of ALL (P <0.0001). RPRM gene, also had a slightly increase in gene expression in treatment ALL group compared to the new cases of ALL (P =0.0412). RPRM gene expression was higher in the normal control group than in the new cases of ALL) P = 0.0372). There was an increase in methylation in both PCDH10 and RPRM genes in new cases of ALL groups compared with treatment ALL group and normal group (P<0.0001 for both genes).

Conclusions: New cases of ALL were associated with high DNA methylation level and low gene expression of PCDH10 and RPRM genes.

Keywords: Acute lymphoblastic leukemia, Methylation, PCDH10, RPRM

Introduction

Acute lymphoblastic leukemia (ALL) is a lymphoid malignancy and hematologic disease that is more prevalent in children and has a favorable prognosis in children than in adults (1). ALL is the most prevalent leukemia and the most common malignancy in childhood, with a peak prevalence between the ages of 2 and 5; it is divided into B and T categories based on the cell line and is more pervasive in boys than in girls. Particularly T-type ALL, which is linked to male predominance. In ALL, differentiation in the lymphocyte cell line is blocked by genetic changes, and proliferation is enhanced in lymphoid

precursors (2, 3). Conrad Waddington introduced the term *epigenetics* in the early 1940s (4). Gene expression is regulated by epigenetic mechanisms at the DNA and chromatin levels. Consequently, studying epigenetic mechanisms in regulating gene expression is vital. In addition to genetic changes, epigenetic changes such as changes in chromatin status, DNA methylation, changes in histone proteins, and the effect of noncoding RNAs contribute to the development of cancer. All of these factors influence gene expression or non-expression (5). DNA methylation is an enzymatic reaction performed by DNA methyl transferase. In CpG-rich regions, these enzymes add a methyl group to the cytosine base (5-methylcytosine (5mC)). Methylated cytosine inhibits gene expression by interfering with the binding of transcription factors (6). DNA methylation contributes to the initiation and progression of cancer, as well as to drug resistance (7). Tumor suppressor genes (TSGs) are frequently inactivated by promoter hypermethylation in numerous types of human cancer (8). Cadherins are a large superfamily of cell adhesion molecules with crucial functions within the body. *Protocadherin-10 (PCDH10)* is a member of this superfamily located at 4q28.3, broadly expressed in all normal adult and fetal tissues. *PCDH10* also plays a role in the formation and function of specific cell-cell connections and may be involved in reducing vascular angiogenesis. Moreover, *PCDH10* is a potential tumor suppressor gene in multiple types of human cancer. Recent research indicates that PCDH10 is epigenetically inactivated in numerous human cancers (9-11). *Reprimo* (RPRM) belongs to the family of genes with a single exon and is found only in vertebrates. This gene, which maps to chromosome 2q23, is a novel, putative tumor suppressor gene frequently silenced by methylation in numerous malignancies. As a new downstream effector of p53-induced cell cycle arrest at the G2/M checkpoint and the tumor signaling pathway, this gene encodes a highly glycosylated cytoplasmic protein that regulates the development and growth of normal cells. In addition, this gene's locus frequently displays an allelic imbalance in certain cancers (12-15). This study aimed to evaluate DNA methylation and gene expression of two tumor suppressor genes in pediatric patients with acute lymphoblastic leukemia, which could be used in the future for therapeutic purposes.

Materials and Methods

Patient's samples

This study included 21 new ALL cases treated at the Department of Pediatrics, Children's Medical Center Hospital (Tehran, Iran). A bone marrow smear and flow cytometer analysis confirmed the diagnosis of ALL, and diagnostic criteria were evaluated according to FAB (French–American–British) classification. The complete patient characteristics are listed in Table I, Figure 1 and Figure 2. At the time of initial diagnosis, prior to the administration of chemotherapy, bone marrow aspirate samples were obtained from ALL patients. All patients participated in the ongoing Multicenter Trial of the GD-2008 ALL protocol for childhood ALL (trial no. NCT00846703); 15 new ALL treated cases were selected as a paired group. For the control group, 18 peripheral blood samples were collected from age- and sex-matched, healthy children of the same age and gender.

Real-time PCR

The TRIzol RNA extraction method was utilized (YTzol RNX-PLUS^{TM} Cat no: EX6101) to isolate total RNA from blood and bone marrow aspirates. RNX- PLUS is a Guanidine/phenol solution used to isolate total RNA from homogenized samples. First, a cold RNX-PLUS solution is added to the sample, followed by chloroform; the mixture is then centrifuged under special

conditions, forming three distinct phases. After transferring the upper phase (aqueous phase) to another tube and washing it with isopropanol and 75% ethanol, the RNA precipitate was dissolved with DEPC Water. Initial RNA quality and quantity evaluations were conducted using gel electrophoresis and NanoDrop (Thermo scientific, Nanodrop one). cDNA Reverse Transcription ParsTous Kit (Easy^{TM} Cdna Synthesis Kit (50) Cat: A101161) was utilized, c-DNA synthesis was performed according to the protocol's guidelines in a total volume of 20 µl with the following thermal profile: 25˚C for 10 min; 47˚C for 60 min; 85˚C for 5 min; finally 4˚C indefinitely. *RPRM* and *PCDH10* gene expression was measured by real-time PCR using forward and reverse primers (Table II). PCR amplification was performed sequentially on a Roche-LightCycler® 96 System; PCR was performed in a total volume of 15 ml containing a 7.5 µl master mix amplicon (Real QPlus 2x Master Mix, SYBER Green Without ROX, cat no: A323402), 1.5 µl mixed Forward and Reverse primer (table II), 4 µl DNase free water and 1µl template. Experiment setup profile was as follows; hold at 95˚C for 15 min for pre-incubation, then PCR stage (40 cycles): denature at 95˚C for 15 s then anneal/extend at 60˚C for 1 min. *GAPDH* gene was used as a housekeeping gene.

Methylation analyze & Bisulfite modification

FAVORGEN kit (blood Genomic DNA Extraction cat no: FABGK001), was utilized to isolate total DNA from blood and bone marrow aspirates.The primary extraction method is columnar DNA extraction. This technology begins with cell lysis, followed by protein removal using proteinase K, DNA binding to a silica substrate, and finally, DNA washing with ethanol. Initial DNA quality and quantity evaluations were conducted using gel electrophoresis and NanoDrop. Genomic DNA was sodium bisulfite modified to selectively convert cytosine to uracil using the EpiTect Bisulfite Kit, Qiagen (Cat No: 59104). Bisulfite conversion thermal cycling conditions were as follows: 95˚C for 5 min; 60˚C for 25 min; 95˚C for 5 min; 60˚C for 85 min; 95˚C for 5 min; 60˚C for 175 min and finally 20˚C indefinitely.

High resolution melting analysis (HRM)

The HRM analysis was performed using a Roche-LightCycler® 96. MS-HRM was performed in a total volume of 15µl containing a 3µl, 5x Hot Firepol Eva Green HRM (5x Hot Firepol Eva Green HRM Master Mix (no ROX), Lot No: 5x HRM0380), 1.5µl (0.1 picomolar was used from each primer). Mixed Forward and Reverse primer (*PCDH10*, Forward: 5'CGGTGGGTGGTGTTTTTG3', Reverse: 5'CTTCAACCTCTAAACCTATAATCT AAAC3'and *Reprimo* primer, Forward: 5'AACGAGTATTATTAGGGTTGGAG3' and Reverse: 5'ACCGTAAAAATTTCCCAAAAACC3'). DNase free water was Variable, and 1µl template and Standard controls (EpiTeCT PCR Control DNA Set (QIAGEN, Cat no :59695). The EpiTect PCR Control DNA Set consists of three different types of human DNA, 1: Amplified human genomic DNA (completely methylated), 2: Completely unmethylated (%0) human genomic DNA-bisulfite converted, 3: Completely methylated (%100) human genomic DNA-bisulfite converted, using 0% and 100% controls. Other controls were prepared in different ratios, (0% ,5%, 25%, 50%,75%,100%). Experiment setup profile was as follows; hold at 95˚C for 15min for pre-incubation, then PCR stage (40 cycles): denature at 95˚C for 10s then anneal/extend at 60˚C for 20s. Melting curve experiment was followed at 95˚C for 60s, 40˚C for 60s, 60˚C for 1s and 95˚C for 1s is continuous with 20 time reading.

Statistical analysis

Statistical analyses were conducted utilizing SPSS 16 and GRAPH pad prism 8. To analyze the gene expression of

PCDH10 and RPRM, the Shapiro-Wilko test was used to examine the normality of data distribution across different groups. All values are presented as mean ± standard error of the mean. The paired t-test for the patient was used to determine statistical significance. The non-parametric Mann-Whitney test was used to compare the gene expression of *PCDH10* between new ALL cases and the control group. Using SPSS, the Chi-square test or Fisher's exact test was utilized to analyze the statistical significance of the HRM technique. The Mann-Whitney test was used to evaluate the effect of promoter methylation on gene expression. A two-way ANOVA determined the significant relationship between age and gender in *PCDH10* and *RPRM* gene expression in ALL patients. To evaluate the relationships between *PCDH10* and RPRM genes in groups, the 2-tailed Spearman correlation coefficients were calculated. The mean difference was calculated using the gene expression difference between groups. The fold change was determined using SPSS software, *P values* less than 0.05 were considered significant.

Ethical consideration

This project was approved by the Ethics Committee of Iran University of Medical science with a code number of IR.IUMS.REC.1398.021

Results

Analysis of the *PCDH10* and *RPRM* genes expression level

As expected from *PCDH10* and *RPRM* genes as suppressive tumor genes in cancer, new ALL cases exhibited a lower *PCDH10* and *RPRM* gene expression rate compared to patients who had received treatment and normal cases. The expression rate of these genes was significantly higher in treated and normal samples than in new ALL cases. $P < 0.05$ was determined by the paired t-test and Mann-Whitney test to indicate that the data was generally significant. The correlation between PCDH10 and RPRM genes (in different groups) was statistically significant ($P < 0.05$) as determined by the Spearman test. Similarly, the comparison of PCDH10 and RPRM gene expression in ALL patients before and after treatment was significant, r = 0.5033 and P = 0.0046, and statistical tests of the relationship between PCDH10 and RPRM gene expression in ALL patients and normal cases yielded the following results: r = 0.5827 and $P \leq 0.0001$ (****). According to the results, it can be concluded that these two genes are highly correlated. In addition, the relationship between *PCDH10* and *RPRM* gene expression, which was related to the age and gender of ALL patients, was investigated using 2-way ANOVA (Table IV). The composition of groups is a mix of age (male and female) and gender (0-3, 3-6, 6-9, and 9-13 years old). The interaction *P-value* was not significant (P = 0.1425, 0.7968 relatively) for either gene. However, a significant correlation between gender and PCDH10 gene expression was observed (P = 0.0235). Thus, there was a significant difference between male and female *PCDH10* expression. However, the correlation between age and PCDH10 gene expression was not statistically significant (P = 0.1682).

DNA methylation analysis of *PCDH10* and *RPRM* genes promoters

For MS-HRM data analysis, the results were divided into two categories: hypo-methylation (methylation<5%) and hyper-methylation (methylation >5%). (Methylation rates in the promoters of *PCDH10* and *RPRM* genes are shown in table V), The chi-square test showed a statistically significant difference in methylation between the PCDH10 and *RPRM* genes in the pre-treatment and post-treatment groups (P =0.033 for *PCDH10* gene, $P < 0.0001$ for *RPRM* gene), the new ALL case, and the healthy control group ($P < 0.0001$ for both genes). These genes were more hypermethylated in the new ALL

case group than in the treatment and healthy control groups. The relationship between methylation and gene expression was analyzed. Real-time Δct data and hypo- or hyper-methylation levels were compared to evaluate potential relationships between gene expression and methylation. Due to the abnormal data distribution, the Mann-Whitney test was utilized; a significant relationship (P <0.0001) was observed between *PCDH10* and *RPRM* gene expression and promoter methylation. Normalized melting curves of HRM analysis were performed for the *PCDH10* and *RPRM* genes and a commercial control at various ratios (Figure 5).

Table I: Demographic characteristics and laboratory variables of the patients

Variables	**New Case (N=21)**
Sex (%)	12 males (57%) 9 females (43%)
Age (years)	1 to 13 (mean:4.5)
Blast (%)	46 to 95 (mean:73.5)
Granulocytes (%)	1 to 44 (mean:10%)
Monocytes (%)	1 to 3 (mean: 1%)
Lymphocytes (%)	3 to 42 (mean:15%)
Karyotype	t (12;21):%9 t (9;22):%5
Type of ALL	Pre-B ALL(%81) T-ALL(%14) ALL/AML(%5)

Table II: Primers designed for Real-Time PCR reaction of PCDH10, RPRM and GAPDH genes.

Primer	**Sequence**	**Tm °**	**CpG product (bp)**
***PCDH10*(F) % 47.62**	**5' TTGTTTGCCTTGCTCTGGATG 3 '**	**59.39**	100
***PCDH10*(R) % 52.38**	**5 'CAGCGATATTCCCCACGAAAG 3 '**	**59.13**	
***RPRM*(F) %61.11**	**5' CTCCCTCGCCCTCTGAAA 3 '**	**58.29**	97
***RPRM*(R) %55**	**5' GCCCTAATCGCCCTCTTCTT 3 '**	**59.53**	
***GAPDH*(F) %50**	**5'AAGGTCGGAGTCAACGGATTTG 3 '**	**60.87**	150
***GAPDH*(R) %50**	**5' GCCATGGGTGGAATCATATTGG 3 '**	**59.44**	

Table III: Analysis of the PCDH10 and Reprimo genes expression level (Results from Real-time PCR), using SPSS software to obtain P-value and fold change in different groups studied.

Dependent Variable	Group(A)	Group(B)	Mean Difference(A-B)	Fold Change	Significant
PCDH10 gene	Before- treatment	After- treatment	3.309	0.10	yes
	After- treatment	Before- treatment	-3.309	9.91	yes
RPRM gene	Before- treatment	After- treatment	2.464	0.18	yes
	After- treatment	Before- treatment	-2.464	5.51	yes
PCDH10 gene	Normal groups	New cases of ALL	-4.731	26.55	yes
	New cases of ALL	Normal groups	4.731	0.03	yes
RPRM gene	Normal groups	New cases of ALL	-1.836	3.57	yes
	New cases of ALL	Normal groups	1.836	0.28	yes

Table IV: Relation of PCDH10 and RPRM genes expression in age and sex parameters

Table IV: 2way ANOVA		***P value***
***PCDH10* gene**	Interaction	0.1425
	Gender	0.0235
	Year	0.1682
RPRM gene	Interaction	0.7968
	Gender	0.6772
	Year	0.4075

Table V: methylation level of PCDH10 & RPRM genes in different groups.

	Total no.	0-5%methylation	5-25%methylation	25-50%methylation	50-75%methylation	75-100% methylation	100% methylation
PCDH10 gene							
Before treatment	15	4(26.6%)	5(33.3%)	3(20%)	2(13.3%)	1(6.6%)	0(0%)
After treatment	15	10(66.6%)	5(33.3%)	0(0%)	0(0%)	0(0%)	0(0%)
New Case	18	4(22.2%)	6(33.3%)	4(22.22%)	3(16.66%)	1(5.55%)	0(0%)
Normal Group	15	14(93.3%)	1(6.66%)	0(0%)	0(0%)	0(0%)	0(0%)
RPRM gene							
Before treatment	15	1(6.6%)	11(73.3%)	3(20%)	0(0%)	0(0%)	0(0%)
After treatment	15	15(100%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
New Case	18	1(5.55%)	12(66.6%)	4(22.2%)	1(5.55%)	0(0%)	0(0%)
Normal Group	15	14(93.3%)	1(6.66%)	0(0%)	0(0%)	0(0%)	0(0%)

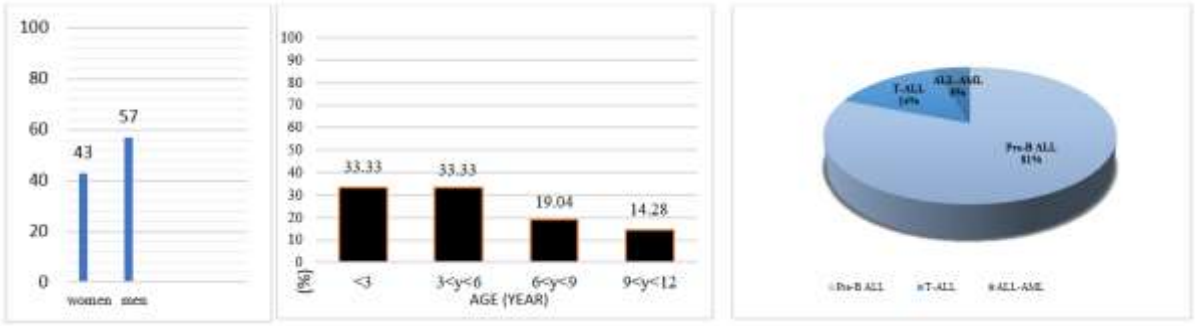

Figure 1: Frequency distribution of gender, age and subgroups of patients in the study (43% of patients were female and 57% were male). In terms of age patients were divided in 4 categories: age less than 3 year 33.33%, age 3-6 year 33.33%, age 6 -9 year 19.04% and age 9 -13 year was 14.28%. In terms of subgroups, pre-B ALL had the highest percentage 81% and next T-ALL 14 % and ALL-AML subgroup 5%.

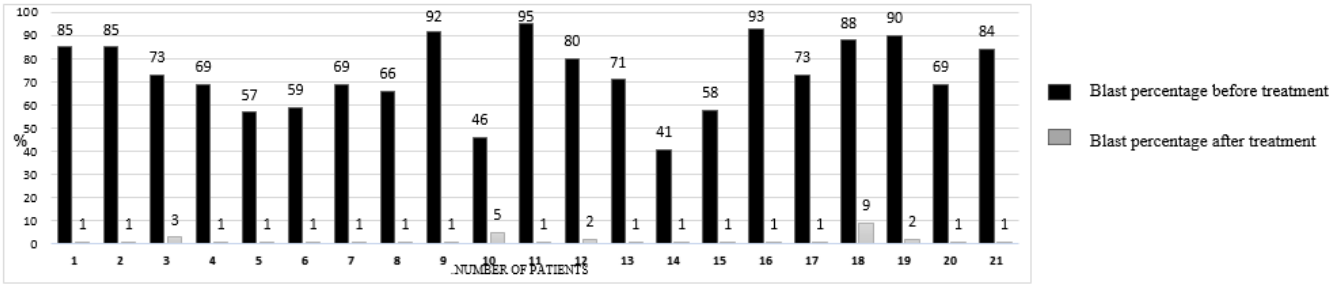

Figure 2: Comparison of blast percentage in patients before and after treatment.

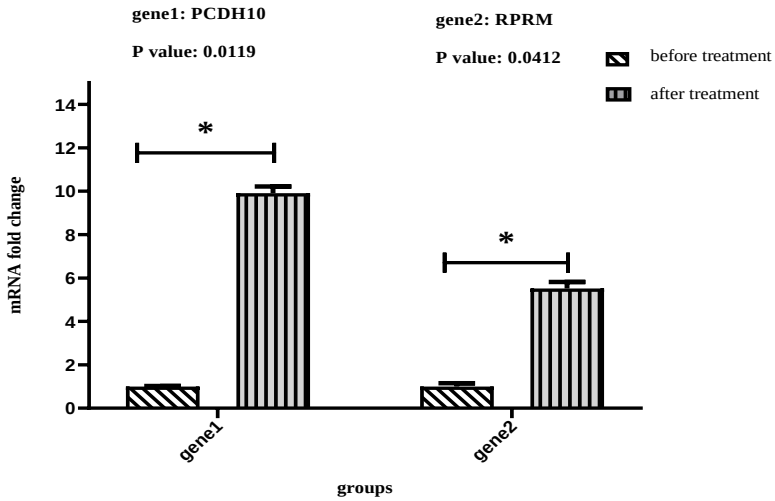

Figure 3: Analysis of *PCDH10* & *RPRM* genes expression rate in patients with ALL, before treatment compared with after treatment. A gene expression differential pattern was found in different groups. Gene expression of *PCDH10* was significantly up regulated in after treatment compared to the before treatment (P = 0.0119), there was a slightly increase in *RPRM* gene expression in the after treatment group compared to the before treatment group (P =0.0412).

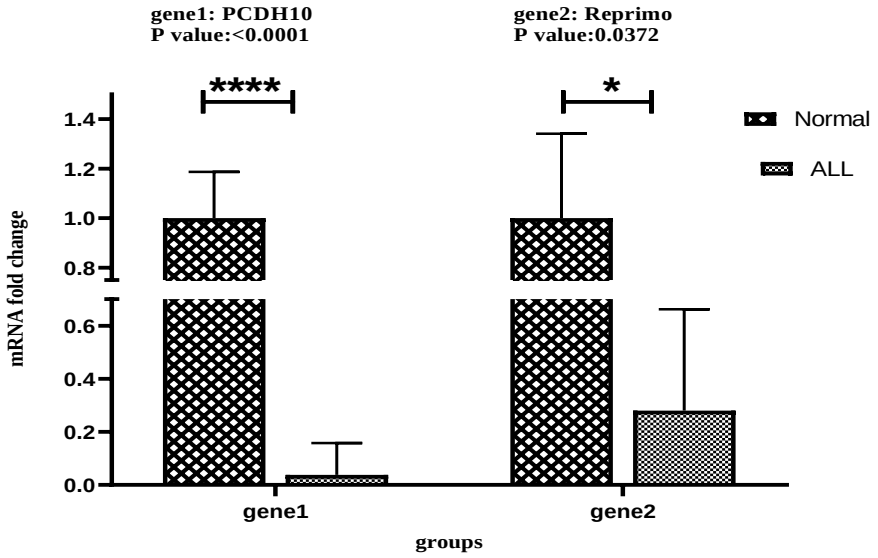

Figure 4: Analysis of *PCDH10 & RPRM* genes expression rate in new cases of ALL compared with normal group. Average ± SEM was plotted for all analyzed, *PCDH10* was higher in the normal group than in the new cases of ALL (P <0.0001). For *Reprimo* gene a significant increase in expression in healthy control group compared with new cases of ALL was observed (P =0.0372).

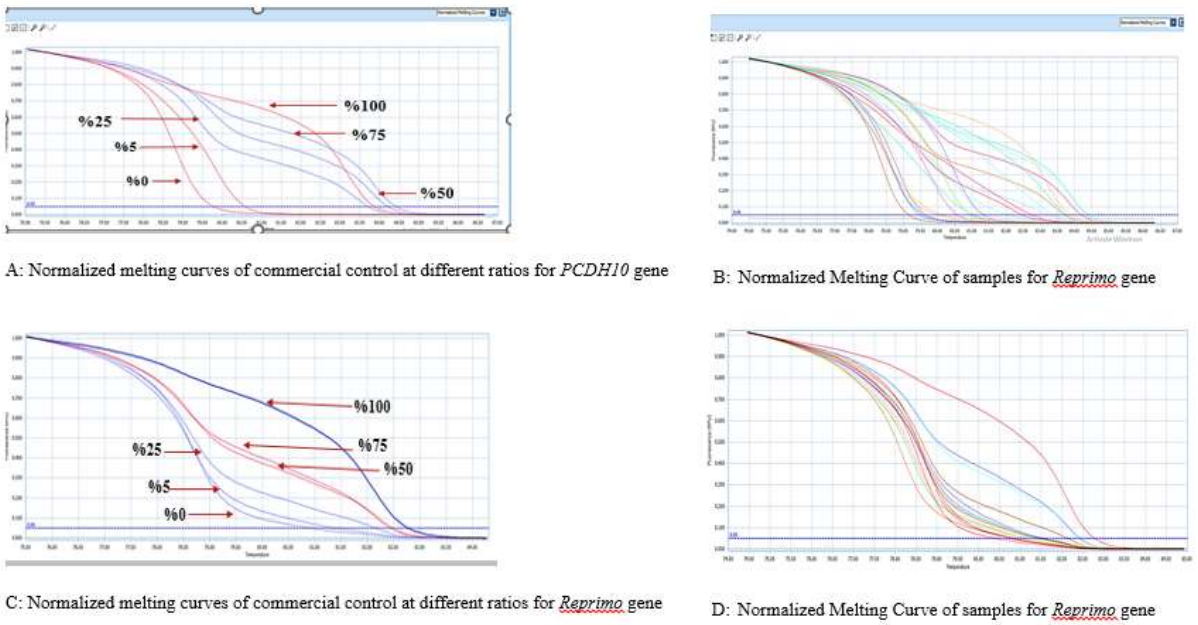

Figure 5: Normalized melting curves of HRM for *PCDH10* and *Reprimo* genes. Standards with different ratios (0%, 25%, 50%, 75%, 100%) of methylated DNA was amplified at the annealing temperature of 60°c.

Discussion

This study investigated two tumor suppressor genes to understand their role in acute lymphoblastic leukemia. According to the published data, *PCDH10* and *RPRM* can act as functional TSGs in multiple cancer cells. *PCDH10* can suppress tumor cell growth, migration, invasion, and colony formation (9). In one study, 215 patients with myeloid and lymphoid leukemia were investigated to evaluate the methylation of the *PCDH10*

gene promoter and its association with resistance to chemotherapy. The *PCDH10* gene promoter hypermethylation was found in ALL. In B-ALL, 81.9% of the *PCDH10* gene promoter was methylated; in T-ALL, the *PCDH10* gene promoter methylation was 80%. Lower methylation was observed in myeloid leukemia patients. High-frequency detection of hypermethylated *PCDH10* gene in both B-ALL and T-ALL suggests that inactivation of this gene likely contributes to the neoplastic stimulation of lymphoid cells in leukemic lymphoblast. Previous research states that *PCDH10* methylation may serve as a marker for chemotherapy response and that the *PCDH10* gene is an epigenetic target in lymphoid leukemia (11). Patients with multiple myeloma were analyzed for methylation of the *PCDH10* gene and its effect on angiogenesis. High methylation and silencing of this gene in patients with multiple myeloma relative to its widespread expression in normal individuals may indicate the role of a tumor inhibitor of this gene in this disease. In addition, the *PCDH10* gene served to inhibit cloning and reduce vascular branching (10). The methylation of the *PCDH10* gene promoter in bladder cancer (BTCC) patients was associated with severe malignancy and a poor prognosis for these patients. This could indicate a separate prognostic factor (16). *PCDH10* methylation was also detected in patients with prostate cancer but not in the control group. *PCDH10* gene methylation was associated with disease invasion and recurrence, suggesting a poor prognosis for patients with prostate cancer after prostatectomy (17). The *PCDH10* gene was analyzed in cervical, gastric, pancreatic, and colorectal cancer. Methylation of the *PCDH10* gene was associated with decreased gene expression in cervical cancer (11). These studies suggest that inactivation of the *PCDH10* gene may be an important step in the progression of cervical cancer and a potential therapeutic target for the disease. The frequency of *PCDH10* methylation was significantly increased in gastric, pancreatic, and colorectal cancers compared to normal tissues (18). According to previous reports, the level of *PCDH10* expression was inversely proportional to the level of methylation. Further aberrant methylation was detected in nasopharyngeal, esophageal, and multiple other carcinomas but not in normal tissues; it is reported that a widely expressed *protocadherin* can also function as a TSG (9). An increase in *RPRM* gene expression inhibits colony formation and anchorage-independent growth. In response to p53 expression, *RPRM* arrests the cell cycle at G2/M, and loss of p53 expression has been associated with increased proliferation and inhibition of apoptosis. This evidence suggests that this gene can inhibit tumor growth (14, 15, 19). Yan-Fang Tao et al. (2015) analyzed the impact of *RPRM* gene methylation in pediatric AML patients. The methylation status of patient samples was higher than that of the control group. Hypermethylated *RPRM* gene promoter samples were associated with decreased survival. Insertion of the *RPRM* gene into cell lines decreased cell proliferation and increased cell apoptosis. Increased *RPRM* expression was also associated with elevated PARP cleavage, a known indicator of apoptosis (14). A study by Saavedra et al. demonstrated a link between the *RPRM* gene and gastric cancer, in which low gene expression was associated with advanced gastric cancer stages (19). Amin Abbasi et al. reported similar findings in patients with gastric cancer; as a result of gene expression, most patients with gastric cancer had reduced expression of the *RPRM* gene compared to healthy individuals (20). Junzhong Lai examined the expression of the *RPRM* gene in gastric cancer (21). The study concluded that the methylation rate in healthy samples was significantly lower than in cancer samples. In addition, they produced cells in which the *RPRM* gene

was silenced, demonstrating that the absence of *RPRM* could result in tumor formation. According to previous studies, the *Reprimo* gene-promoter region was significantly hypermethylated in GC tissues, plasma, and cell samples, allowing it to be detected as a diagnostic biomarker for GC in plasma or serum samples from patients with GC (22). In addition, Wong et al. (2005) examined the status of the *Reprimo* gene in cervical carcinoma, which showed decreased expression and increased methylation in patients compared to the control group; *Reprimo* methylation was a unique event in head and neck carcinoma (23). In pancreatic cancer, Sato et al. (2006) observed that patients with methylation in the *Reprimo* gene promoter had genetic instability and a poorer prognosis than those without methylation (24). In addition, the CpG of the *Reprimo* gene was hypermethylated in breast cancer compared to normal breast tissue, and interestingly, ERα(+) tumors displayed a higher degree of methylation than ERα(-)(12). Leukemia, a blood cancer, is the most prevalent form of cancer in children. Approximately 30% of childhood cancers are leukemias, with ALL being the most prevalent (1). It appears that epigenetic changes may play a significant role in the disease's onset. These alterations are frequently caused by the inhibition of tumor suppressor genes or an increase in the expression of oncogenic genes. These changes in gene expression are impacted and can even lead to cancer development. Additionally, epigenetic changes may play a role in cancer development, and because these changes are reversible, there are drugs known as epi-drugs that can affect these epigenetic changes. It is significant because it can serve as a therapeutic target (25-27) .One of the changes in cancers results from the increased activity of DNA methylation enzymes, which alters the DNA methylation pattern and may result in a decrease (hypomethylation) or increase (hypermethylation) in the methylation rate (28). In the present study, the expression of the PCDH10 and RPRM genes was higher in ALL patients after treatment than in ALL patients before treatment. The expression of the studied genes was significantly higher in the healthy control group than in the ALL group. Using MS-HRM, this study investigated the methylation of genes. A significant difference was observed between the methylation of genes before and after treatment in new ALL cases and the control group. In addition, the relationship between promoter methylation and gene expression was investigated. This study suggests gene promoter methylation may influence gene expression and patient characteristics based on gene expression and methylation percentage. The expression of the *PCDH10* gene was lowest in patient numbers 15, 10, and 18, respectively. Notably, these patients (10 and 18) had a high blast percentage after treatment. Intriguingly, the methylation rate of patient 15 ranged between 75 and 100%. Consequently, methylation of the gene has been linked to its expression. Patients 17, 2, and 16 had the highest expression of the *PCDH10* gene; all were females with a blast percentage of 1% after treatment. Regarding the *RPRM* gene, patient number 15 and patients 10-14 had the lowest gene expression. The patients with the highest *RPRM* gene expression were 1, 2, and 21, respectively. After treatment, the blast percentage of these patients was 1%. Patient number 8 exhibited the highest level of methylation. There was no correlation between gene expression and methylation and the characteristics of patients. The findings of this study were consistent with previous research. According to this study, the *PCDH10* and *RPRM* genes may play a role in tumor suppression in patients with acute lymphoblastic leukemia. Methylation of the *PCDH10* and *Reprimo* promoters has been identified in several human cancers. In ALL, expression and methylation of

these genes were investigated due to their role as novel tumor suppressors and their significance in disease prognosis, as determined by prior research. In the present study, we demonstrate that aberrant methylation of *PCDH10* and *Reprimo* in acute lymphoblastic leukemia is consistent with previous research. According to this study, the *PCDH10* and *RPRM* genes may play a role in tumor suppression in patients with acute lymphoblastic leukemia. This study may provide new evidence that the promoters of the *PCDH10* and *RPRM* genes play a role in ALL patients via multiple signaling pathways, including the EGFR/AKT pathway. Further research is proposed to demonstrate that *PCDH10* and *RPRM* gene methylation may be significant treatment markers for ALL.

Conclusion

In conclusion our findings indicate that *PCDH10* and *RPRM* methylation may function as tumor suppressor genes in ALL cells. These findings also support further investigation into the possible role of *PCDH10* and *RPRM* gene expression and methylation in ALL patients. However, investigations on numerous pediatric ALL patients are necessary to validate the current findings.

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Conflict of interest

The authors declare no conflict of interest.

References

1. Yu R-S, Zhang W-M, Liu Y-Q. CT diagnosis of 52 patients with lymphoma in abdominal lymph nodes. World J Gastroenterol 2006; 12(48): 7869-7873.
2. Eberhardt F, Köhler C, Winter K, Alef M, Kiefer I. Sonographically detectable changes in abdominal lymph nodes in dogs with malignant lymphoma. Evaluation with special consideration of the Solbiati-Index. Tierarztl Prax Ausg K Kleintiere Heimtiere 2015; 43(5): 309-316.
3. Mori T. Childhood lymphoma. Rinsho Ketsueki 2016; 57(10): 2285-2293.
4. Kebede AA, Bekele F, Assefa G. Abdominal Lymphoma: Imaging Work Up Challenges and Recommendationes in Resorce Limited Setup. Ethiopian Med J 2014; 52(4): 197-206.
5. Garciaz S, Coso D, Brice P, Bouabdallah R. Hodgkin and non-Hodgkin lymphoma of adolescents and young adults. Bull Cancer 2016; 103(12): 1035-1049.
6. Thanarajasingam G, Bennani-Baiti N, Thompson CA. PET-CT in staging, response evaluation, and surveillance of lymphoma. Curr Treat Options Oncol 2016; 17(5): 1-9.
7. Diefenbach CS, Connors JM, Friedberg JW, Leonard JP, Kahl BS, Little RF, et al. Hodgkin lymphoma: current status and clinical trial recommendations. J Natl Cancer Inst 2017; 109(4): 249-255.
8. Zhou Q-T, Zhu H, He B. Clinical analysis of lymphoma with chest involvement: report of 25 cases. Zhonghua Nei Ke Za Zhi 2009; 48(10): 846-849.
9. Haaga JR, Boll D. Computed Tomography & Magnetic Resonance Imaging of The Whole Body E-Book: Elsevier Health Sciences; 2016.
10. Smith-Bindman R, Miglioretti DL, Johnson E, Lee C, Feigelson HS, Flynn M, et al. Use of diagnostic imaging studies and associated radiation exposure for patients enrolled in large integrated health care systems, 1996-2010. JAMA 2012; 307(22): 2400-2409.
11. Miglioretti DL, Johnson E, Williams A, Greenlee RT, Weinmann S, Solberg LI, et al. The use of computed tomography in pediatrics and the associated radiation exposure and estimated cancer risk. JAMA Pediatr 2013; 167(8): 700-707.
12. De González AB, Mahesh M, Kim K-P, Bhargavan M, Lewis R, Mettler F, et al. Projected cancer risks from computed

tomographic scans performed in the United States in 2007. Arch Intern Med 2009; 169(22): 2071-2077.
13. Slovis TL. CT and computed radiography: the pictures are great, but is the radiation dose greater than required? AJR Am J Roentgenol 2002; 179(1): 39-41.
14. Brody AS, Frush DP, Huda W, Brent RL, Radiology So. Radiation risk to children from computed tomography. Pediatrics 2007; 120(3): 677-682.
15. Brenner DJ, Elliston CD, Hall EJ, Berdon WE. Estimated risks of radiation-induced fatal cancer from pediatric CT. AJR Am J Roentgenol 2001; 176(2): 289-296.
16. De Gonzalez AB, Salotti JA, McHugh K, Little MP, Harbron RW, Lee C, et al. Relationship between paediatric CT scans and subsequent risk of leukaemia and brain tumours: assessment of the impact of underlying conditions. Br J Cancer 2016; 114(4): 388-394.
17. Mathews JD, Forsythe AV, Brady Z, Butler MW, Goergen SK, Byrnes GB, et al. Cancer risk in 680 000 people exposed to computed tomography scans in childhood or adolescence: data linkage study of 11 million Australians. BMJ 2013; 346: f2360-2365.
18. Polites SF, Mohamed MI, Habermann EB, Homme JL, Anderson JL, Moir CR, et al. A simple algorithm reduces computed tomography use in the diagnosis of appendicitis in children. Surgery 2014; 156(2): 448-454.
19. Smith-Bindman R, Aubin C, Bailitz J, Bengiamin RN, Camargo Jr CA, Corbo J, et al. Ultrasonography versus computed tomography for suspected nephrolithiasis. N Engl J Med 2014; 371(12): 1100-1110.
20. Dann EJ, Berkahn L, Mashiach T, Frumer M, Agur A, McDiarmid B, et al. Hodgkin lymphoma patients in first remission: routine positron emission tomography/computerized tomography imaging is not superior to clinical follow-up for patients with no residual mass. Br J Haematol 2014; 164(5): 694-700.
21. Tomé A, Costa F, Schuh J, Monteiro L, Monteiro A, Botelho de Sousa A. No benefit of routine surveillance imaging in Hodgkin lymphoma. Br J Haematol 2015; 168(4): 613-614.
22. Voss SD, Chen L, Constine LS, Chauvenet A, Fitzgerald TJ, Kaste SC, et al. Surveillance computed tomography imaging and detection of relapse in intermediate-and advanced-stage pediatric Hodgkin's lymphoma: a report from the Children's Oncology Group. J Clin Oncol 2012; 30(21): 2635-2640.
23. Cheson BD, Fisher RI, Barrington SF, Cavalli F, Schwartz LH, Zucca E, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. J Clin Oncol 2014; 32(27): 3059-3068.
24. Adedayo AA, Igashi JB, Mshelbwala PM, Nasir AA, Ameh EA, Adeniran JO. Accuracy of ultrasonography in the evaluation of abdominal masses in children in Nigeria. Afr J Paediatr Surg 2019; 16(1-4): 1-5.
25. Kebede AG, Nigussie Y. Ultrasound evaluation of abdominal masses in Ethiopian child patients. Trop Doct 2011; 41(3): 157-159.
26. Crabtree AC, Spangler E, Beard D, Smith A. Diagnostic accuracy of gray-scale ultrasonography for the detection of hepatic and splenic lymphoma in dogs. Vet Radiol Ultrasound 2010; 51(6): 661-664.
27. Rahmani G, McCarthy P, Bergin D. The diagnostic accuracy of ultrasonography for soft tissue lipomas: a systematic review. Acta Radiol Open 2017; 6(6): 2058460117716704-058460117716709.
28. Hashemi A, Nafisi MR, Ghilian R, Shahbaz S, Dehghani TA. The Accuracy of Ultrasonography in Compared with Computed Tomography in Detection of Anterior Mediastinal Neoplasms. Iran J Ped Hematol Oncol 2012; 2(1): 6-10.