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Increased levels of *cyclin D1* negatively impacts on acute lymphoblastic leukemia overall survival

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Abstract

Background: Cyclin D1 is a protein essential for transition from G_1 to S phase during cell cycle progression, which has an oncogenic potential and is highly expressed in several human malignancies. However, in view of the heterogeneity of the findings in the literature, the prognostic value of *cyclin D1* expression still needs to be validated in different cohorts of adult acute lymphoblastic leukemia (ALL) patients.

Methods: Bone marrow samples from 13 healthy donors and 45 adult patients with acute lymphoblastic leukemia were included. *Cyclin D1* gene expression was evaluated by quantitative PCR. For statistical analysis, Mann–Whitney test, Fisher's exact test, Chi-squared test and Cox regression were used, as appropriate. All *p* values were two-sided with a significance level of 5%.

Results: *Cyclin D1* mRNA levels were similar between primary cells from ALL patients and healthy donors. In ALL patients, high *cyclin D1* expression was associated with older age at the diagnosis, presence of BCR-ABL1, and lower white blood cell counts. Importantly, increased *cyclin D1* expression was an independent factor that predicted worse overall survival in our adult ALL cohort.

Conclusion: Increased levels of *cyclin D1* negatively impacted on ALL survival outcome, suggesting that this gene is involved in the malignant phenotype of ALL.

Keywords: Cyclin D1, CCND1, Acute lymphoblastic leukemia, Prognosis

Background

Acute lymphoblastic leukemia (ALL) is a heterogeneous group of neoplasm characterized by aberrant clonal proliferation and accumulation of B or T lymphoid immature cells in hematopoietic tissues, which impairs the normal hematopoiesis [1]. Adult ALL is associated with higher rates of death and relapse [2]. Cyclin D1 (CCND1) is an essential protein in the transition from G_1 to S phase during cell cycle progression [3], which has an oncogenic potential and is highly expressed in several human malignancies [3, 4]. In addition to its function in cell cycle control, cyclin D1 plays a role in the gene transcription regulation, cell migration, differentiation and energy balance that contributes to development and maintenance of cancer phenotype [3, 5].

The aim of the present study was to investigate the *cyclin D1* (*CCND1*) mRNA expression and its association with clinical and laboratorial characteristics, and its impact on overall survival in a cohort of adult ALL patients.

Methods

Primary samples

Bone marrow samples were collected from 13 healthy donors from related bone marrow transplantation (median age 29 years [range 15–51]). Bone marrow or peripheral blood samples were collected from 45 patients with acute lymphoblastic leukemia (median age 35 years

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[range 18–79]) at the time of diagnosis ($n = 40$) or relapse ($n = 5$), followed up in the Clinical Hospital of our Institution. The median percentage of blasts in peripheral blood and bone marrow samples were 68% (range 50–88, $n = 8$) and 86% (range 26–100, $n = 37$), respectively. The study was approved by the Ethics Committee of the institution and written informed consent was obtained from all subjects who participated in this study. Patient's characteristics are described in Table 1.

Quantitative PCR (qPCR) analysis

Total RNA was obtained from total nucleated bone marrow cells, after removal of erythrocytes by hemolysis, using TRIzol reagent (Thermo Fisher Scientific; Carlsbad, CA, USA). The cDNA was synthesized from 1 μg of RNA using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Quantitative PCR was performed using PowerUp Sybr Green master mix (Thermo Fisher Scientific) in a 7500 Real Time PCR System (Thermo Fisher Scientific) with specific primers for *cyclin D1* (*CCND1;* FW: CTGGGTGTCCTACAAATG; RV: AGCGGTCCAGGT AGTTCAT) and for the reference gene *β-actin* (*ACTB*; FW: AGGCCAACCGCAAGAAG; RV: ACAGCCTGGATA GCAACGTACA) using a total of 120 ng of cDNA for each replica for *CCND1* and *ACTB* genes. *ACTB* was the reference gene. A negative 'No Template Control' was included for each primer pair. Two replicas were run on the same plate for each sample. The relative quantification value was calculated using the equation $2^{-\Delta\Delta CT}$.[6].

Statistical analysis

Statistical analyses were performed using GraphPad Instat 5 (GraphPad Software Inc., San Diego, CA, USA), Stata Statistic/Data Analysis 14.1 (Stata corporation, USA) and statistical package for the social sciences (SPSS) 19.0. Mann–Whitney test was used for measured factors. Fisher's exact test or Chi-squared test was used for categorical factors. Univariate and multivariate proportional hazard regression analysis was performed for potential prognostic factors for overall survival using Cox regression. Overall survival was defined from time of sampling to date of death or last seen. Patients included in the study at the time of diagnosis ($n = 40$) were included in the survival analysis. All p values were two-sided with a significance level of 5%.

Results

Cyclin D1 expression negatively impacts on ALL overall survival

Cyclin D1 mRNA levels did not differ between primary cells from healthy donors and ALL patients (median 1.38 [0.25–2.71] vs. 1.00 [range 0.06–41.09]; $p = 0.49$; Fig. 1a). When ALL patients were stratified according to molecular and immunophenotypic characteristics, *cyclin D1* expression was significantly higher in *BCR-ABL1*-positive compared with *BCR-ABL1*-negative ALL patients (1.81 [0.16–8.83] vs. 0.72 [0.06–41.09], $p < 0.05$;

Table 1 Clinical and laboratory data from acute lymphoblastic leukemia patients

Characteristics	Total patients ($n = 45$)	*Cyclin D1*[a]		*p*-value
		Low expression ($n = 20$)	High expression ($n = 20$)	
Age, years; median (range)	27 (18–79)	22 (18–67)	38 (18–79)	**0.022**
Gender				
Male, n (%)	30 (66.7)	14 (70)	13 (65)	
Female, n (%)	15 (33.3)	6 (30)	7 (35)	0.49
Immunophenotype				
B-ALL, n (%)	35 (79.5)	14 (70)	18 (90)	0.114
T-ALL, n (%)	9 (20.5)	6 (30)	2 (10)	
BCR-ABL1 positive, n (%)				
Positive, n (%)	10 (22.2)	1 (5)	7 (35)	
Negative, n (%)	35 (77.8)	19 (95)	13 (65)	**0.018**
Peripheral blood counts, median (range)				
Hemoglobin, g/dL	9.1 (5.2–15.8)	7.9 (5.2–15.2)	9.3 (5.3–15.8)	0.235
WBC, $\times 10^9$/L	12.2 (0.8–549.5)	23.8 (3.2–549.5)	8.2 (0.8–61.8)	**0.013**
Platelets, $\times 10^9$/L	38 (4–433)	29 (4–162)	38 (7–433)	0.419
LDH, median (range); U/L	1440 (245–13090)	2625 (270–13090)	1410 (245–6848)	0.09

Abbreviations: *ALL* acute lymphoblastic leukemia, *T-ALL* precursor T-acute lymphoblastic leukemia, *B-ALL* precursor B-acute lymphoblastic leukemia, *BCR-ABL1* breakpoint cluster region-abelson 1, *WBC* white blood cell, *LDH* lactic dehydrogenase

[a]Patients with ALL whose sample were collected at diagnosis ($n = 40$) were included in the analysis

Statistically significant p values are highlighted in bold

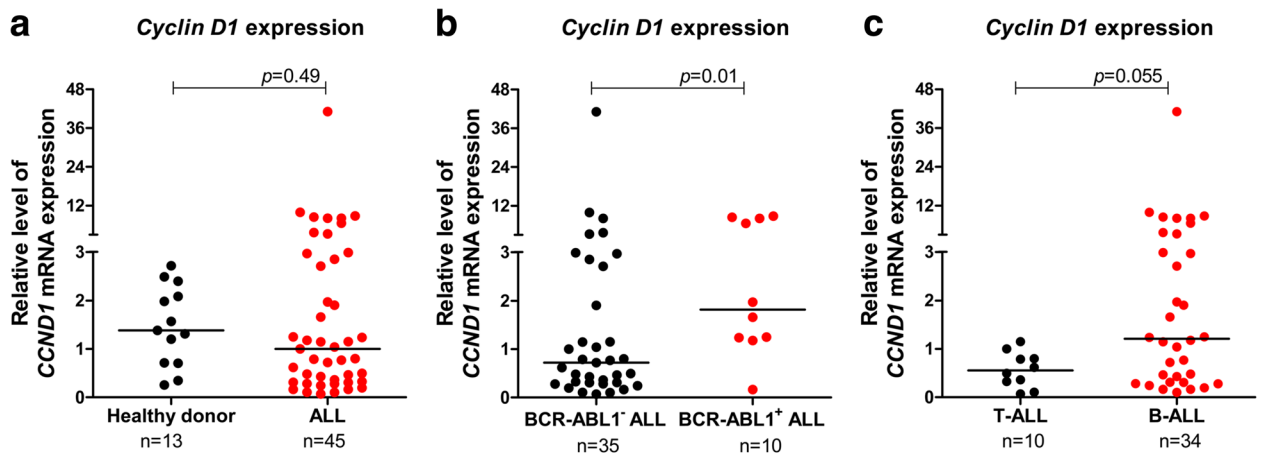

Fig. 1 *Cyclin D1* expression in primary samples from adult acute lymphoblastic leukemia (ALL) and healthy donors. Quantitative RT-PCR analysis of *cyclin D1* (*CCND1*) mRNA levels in samples from healthy donors and ALL patients (**a**) and in ALL patients stratified according to *BCR-ABL1* status (**b**) and immunophenotype (**c**). The *ACTB* gene was used as the reference gene and a healthy donor was used as a calibrator sample. Horizontal lines indicate medians. The numbers of subjects studied are indicated in the graph. The *p*-values are indicated; Mann-Whitney test

Fig. 1b). *Cyclin D1* expression did not significantly differ between B-ALL and T-ALL patients (1.20 [0.10–41.09] vs. 0.55 [0.06–1.15]; $p = 0.055$; Fig. 1C). High *cyclin D1* expression was associated with older age at the diagnosis, presence of *BCR-ABL1*, and lower white blood cell (WBC) counts in ALL patients (Table 1). Importantly, increased levels of *cyclin D1* and age were independent factors that predicted worse overall survival in our adult ALL cohort (Table 2).

Discussion

In the present study, we found no difference between the *Cyclin D1* expression in primary cells from ALL patients and healthy donors. Using qPCR and Western blot assays, Wang et al. [7] observed an increased levels of *cyclin D1* mRNA and protein expression in peripheral blood or bone marrow mononuclear cells of 60 ALL adult patients compared to 30 healthy donors. In contrast, Taniguchi et al. [8] did not find *cyclin D1* overexpression in any of the 11 ALL patients evaluated.

We found that *cyclin D1* expression independently predicted overall survival in our adult ALL cohort. Another study observed a positive correlation between *cyclin D1* expression and the blast cell count in peripheral blood and bone marrow of ALL patients, but no correlation between *cyclin D1* expression and WBC counts, hemoglobin level or platelet counts was found [9]. On the other hand, Elsayed et al. reported that *cyclin D1* expression was not associated with response to chemotherapy and overall survival in 78 newly diagnosed adult and pediatric ALL patients [10]. High *cyclin D1* mRNA levels had already been associated with poorer prognosis and relapse in childhood ALL [11, 12]. In view of the heterogeneity of the findings in the literature, the evaluation of the prognostic impact of *cyclin D1* expression in ALL survival outcomes is still an open field for further investigation.

In our study, we found an association between the expression of *cyclin D1* and the presence of BCR-ABL1: 8 out of 40 (20%) patients with ALL at diagnosis presented BCR-ABL1, among these, only one did not present high *cyclin D1* expression. BCR-ABL1 is a potent oncogene that induces high rates of proliferation and cell cycle progression [13, 14]. Several studies have reported the

Table 2 Univariate and multivariate analyses of overall survival for patients with acute lymphoblastic leukemia[a]

Factor	Univariate analysis			Multivariate analysis		
	HR[b]	(95% C.I.)	*p*	HR[b]	(95% C.I.)	*p*
Age	1.04	1.02–1.07	< **0.001**	1.04	1.02–1.08	< **0.001**
BCR-ABL1 (positive vs. negative)	1.09	0.44–2.73	0.852	0.68	0.26–1.76	0.431
White blood cell count ($\times 10^9$/L)	0.998	0.995–1.002	0.542	1.0007	0.997–1.004	0.702
Cyclin D1 expression	1.07	1.01–1.13	**0.019**	1.07	1.01–1.15	**0.026**

Abbreviations: ALL, acute lymphoblastic leukemia; HR, hazard ratio; *BCR-ABL1*, breakpoint cluster region-abelson 1
[a]Patients with ALL whose sample were collected at diagnosis ($n = 40$) were included in survival analysis
[b]Hazard ratios > 1 indicate that increasing values for continuous variable or the first factor for categorical variable has the poorer outcome
Statistically significant *p* values are highlighted in bold

relationship between BCR-ABL1 and cyclin D1 expression, mainly in chronic myeloid leukemia [14, 15]. Although BCR-ABL1 is recognized as a marker of poor prognosis in ALL [16, 17], BCR-ABL1 was not a prognostic factor in our cohort; the low number of BCR-ABL1 positive ALL cases probably interfered with the survival analysis.

Conclusion

In conclusion, our data indicates that *cyclin D1* mRNA levels were similar between primary cells from ALL patients and healthy donors. Increased levels of *cyclin D1* negatively impacted on adult ALL overall survival, suggesting the involvement of this gene in the malignant phenotype of ALL.

Abbreviations

ACTB: β-actin; ALL: Acute lymphoblastic leukemia; B-ALL: Precursor B-acute lymphoblastic leukemia; BCR-ABL1: Breakpoint cluster region-abelson 1; CCND1: Cyclin D1; HR: Hazard ratio; LDH: Lactic dehydrogenase; qPCR: Quantitative polymerase chain reaction; RT-PCR: Reverse transcription polimerase chain reaction; T-ALL: Precursor T-acute lymphoblastic leukemia; WBC: White blood cell

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Availability of data and materials

Please contact author for data requests.

Authors' contributions

JCF designed the study and experiments, performed all the experiments, manuscript preparation, completion and final approval. APNRA contributed with sample selection, inputs on overall design of study, manuscript editing and final approval. JLC-S performed statistical analysis, contributed with inputs on overall design of study, manuscript editing and final approval. RS-R and BAF contributed with inputs on overall design of study, manuscript editing and final approval. BPS and EMR participated in the interpretation of manuscript data, in manuscript editing and final approval. JAM-N participated in overall design of study and experiments, statistical analyses, manuscript preparation, editing, completion and final approval. FT was the principal investigator and participated in overall design of study and experiments, statistical analyses, manuscript preparation, editing, completion and final approval. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the University of São Paulo at Ribeirão Preto Medical School in accordance with the Helsinki Declaration. Written informed consent was obtained from all healthy donors and ALL patients who participated in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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