High Expression of ECM1 At Diagnosis Predicated Poor Prognosis in Adult B-Cell Acute Lymphoblastic Leukemia

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Abstract

Identifying new prognostic biomarkers and selecting treatment regimens based on them can improve outcomes of adult patients with B-cell acute lymphoblastic leukemia (B-ALL). Extracellular matrix protein 1 (ECM1) is a glycoprotein involved in many biologic processes. Here we aimed to determine the expression and the prognostic value of ECM1 in B-ALL. One hundred and six newly-diagnosed B-ALL patients (seventy-one adult patients and thirty-five children patients) were enrolled from March 2013 to December 2018. The ECM1 transcript levels were significantly different among different disease state. Among 71 adult B-ALL patients, ECM1-high group has a higher initial platelet count ($p=0.004$) more BCR-ABL fusion positive patients ($p=0.004$) and poor risk patients ($p=0.021$) than ECM1-low group, whereas no significant relationship with any other clinical characteristics was observed. ECM1-high group had significantly lower 3-year OS (11.4% vs. 36.1%, $p=0.015$) and 3-year RFS (9.7% vs. 29.4%, $p=0.047$) compared to ECM1-low group. In the multivariate Cox regression model, high ECM1 expression was an independent adverse prognostic factor for OS (HR=3.0(1.4-6.6), $p=0.007$). Our data demonstrated that the expression of ECM1 could serve as a novel prognostic biomarker for adult patients with B-ALL.

Introduction

B-cell acute lymphoblastic leukemia (B-ALL) accounts for approximately 15% to 20% of all adult acute leukemias. Despite much progress over the last several decades, the therapeutic outcome in adult B-ALL remains unsatisfactory, with a long-term disease-free survival (DFS) of 40% to 45%. This is in contrast to childhood B-ALL where DFS of >90% is routinely achieved. Although some indicators, including age, WBC count, cytogenetic abnormalities, time to achieve complete remission (CR), and measurable residual disease (MRD) have been confirmed as risk factors in adult ALL^{1,2}, outcomes of patients without adverse prognostic factors remain clinically heterogeneous. Therefore, the identification of novel prognostic biomarker is necessary to choose the appropriate treatment regimens and improve the survival.

The human extracellular matrix 1 (ECM1) gene maps to 1q21 and encodes an 85 kDa glycoprotein³. ECM1 germline mutations lead to lipoid proteinosis⁴. Moreover, ECM1 also play important roles in angiogenesis, in mineralization, in tumor progression and in tumor metastasis⁵⁻⁷. ECM1 is essential for the ability of breast cancer cells to matrix attachment, invasion, and spontaneous metastasis⁸. Besides, ECM1 can promote cell metastasis and glucose metabolism in gastric cancer⁹. The expression level of ECM1 was associated with metastatic potential of hepatocellular carcinoma and was a novel independent prognostic factor of overall survival and disease-free survival¹⁰. To date, there is no information on the prognostic value of ECM1 in adult B-ALL.

In the current study, the expression level of ECM1 was evaluated in B-ALL patients with different disease states and normal control. The relationship between ECM1 expression level and the clinical characteristics of 71 adult B-ALL patients were next examined. Furthermore, potential prognostic of ECM1 in adults B-ALL were analyzed based on the survival data.

Materials And Methods

Patients and treatment strategy

Bone marrow samples from newly-diagnosed (ND) B-ALL patients (n=106), completion remission (CR) B-ALL patients (n=73), refractory or relapse (RR) patients (n=25) were enrolled from the First Affiliated Hospital of Zhengzhou University between March 2013 to December 2018. In addition, thirty-six healthy donors in the same period were enrolled in the study as the control group. Among the 106 newly-diagnosed B-ALL patients, data of 71 adult patients (age 14 years or older) were collected for further analysis.

Patients with Philadelphia chromosome negative (Ph-) B-ALL were treated with 1-2 cycles of induction chemotherapy (VDCLP, including vincristine, daunorubicin, cyclophosphamide, L- asparaginase and prednisone/dexamethasone). Patients with Philadelphia chromosome positive (Ph+) B-ALL were treated with tyrosine kinase-inhibitor (TKI) therapy in combination with the same induction chemotherapy but without L- asparaginase. Patients who achieved CR received consolidation chemotherapy alternating with high-dose methotrexate or cytarabine therapy until they received an allogeneic hematopoietic stem cell transplantation (allo-HSCT) or completed six additional chemotherapy cycles. Maintenance therapy included 6-mercaptopurine and methotrexate. Patients with Ph+ B-ALL also received post-transplant or post-consolidation TKI therapy. Central nervous system prophylaxis was performed for all patients by intrathecal administration of triple agents (methotrexate, cytarabine, and hydrocortisone; ≥ 6 times in total). Thirty-three patients received allo-HSCT: 23 (69.7%) from a HLA-haplotype-mismatched related donor and 10 (30.3 %) from an HLA-identical sibling donor. The patients were followed until death, loss to follow-up or until December 2019.

Immune phenotype, molecular and cytogenetic analyses, and MRD

Immune phenotypes were identified as previously reported². BCR-ABL fusions, MLL rearrangement, IKZF1 deletions and WT1 transcripts were detected as previously reported¹¹. Cytogenetic analyses were performed by using standard G-banding. MRD was quantified as previously reported¹². MRD positive was defined as subjects where $\geq 0.1\%$ of the cells displayed a leukemia-associated aberrant immune phenotype (LAIPs).

Isolation of RNA and cDNA synthesis and real-time quantitative PCR (RQ-PCR)

The extraction of total RNA and the synthesis of c-DNA were performed as previously described¹³. The 10 μ l PCR reaction mixture contained 5 μ l 1 $\times$ TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 400 nM primers, 250 nM fluorescent probes, and 150–500 ng cDNA. The primers and probe sequences are shown in Table 1. The RQ-PCR was performed as previously described¹⁴.

Endpoints and statistical analyses

Overall survival (OS) was measured from the date of diagnosis to the date of death from any cause. Relapse free survival (RFS) was defined as the time from the date of first CR to first hematologic or extramedullary relapse or death from any cause. Observations were censored at the date of last contact or December, 2019 when no events were observed.

Categorical variables were compared by Chi-square analysis or Fisher exact test and continuous variables by Mann-Whitney U test or Student t-test. Kaplan-Meier methods were used to estimate the probability of OS and RFS over time, providing median and 95% confidence intervals (CIs). Cox proportional hazard regression models were used to determine associations between ECM1 expression level and other variables with OS and RFS. All data analyses were performed using the SPSS software version 21.0 (Chicago, IL, USA). Difference with $p < 0.05$ was considered statistically significant.

Results

Abnormal ECM1 expression in B-ALL patients

The ECM1 transcript levels in ND (median 101.3%, range 0-5411.7%) and RR B-ALL patients (median 39.1%, range 0-6365.5%) were significantly increased than it in normal control (median 2.8%, range 0-4.97%) ($p < 0.01$). While the ECM1 transcript level was significantly decreased in CR patients (median 3.3%, range 0-196.9%) compared with the ND and RR B-ALL patients ($p < 0.01$). Besides, there was no statistically significant difference in ECM1 transcript levels between children (median 71.8%, range 0-5411.7%) and adult patients (median 106.3%, range 0-2431.6%) ($p \geq 0.05$) (Fig.1).

Patient characteristics

Then the data of 71 adult patients (age 14 years or older) were collected for further analysis. The baseline characteristics are summarized in Table 2. The median age was 37 years old. And 62.9% ($n=44$) patients were male. The median WBC count, hemoglobin, and platelet count at diagnosis were 17.5 (range 1.6–586.2) $\times 10^9$ /L, 82 (range 28.9-158) g/L and 56 (range 4-338) $\times 10^9$ /L respectively. Among them, 51 patients were common-B-ALL, 10 patients were progenitor B-ALL and 10 patients were precursor-B-ALL. Regarding the molecular biological features, 32 patients had BCR-ABL fusion (Ph+), 4 patients had MLL rearrangement. According to the cytogenetic risk, 57.5% ($n=41$) patients were poor risk. 24 patients were MRD positive at the end of the induction therapy. 53.5% ($n=38$) patients only received chemotherapy and the other patients received allo-HSCT.

High expression of ECM1 at diagnosis predicted poor outcomes in adult B-ALL patients

To assess the clinical significance of ECM1 in adult B-ALL patients, we next examined the relationship between ECM1 expression level and the clinical characteristics of 71 patients. Patients were grouped into ECM1-Low or ECM1-High according to the median baseline ECM1 expression levels. As shown in Table 2, compared with ECM1-Low group, the patients in ECM1-High group were more likely to have higher platelet

number ($p=0.004$), BCR-ABL fusion ($p=0.004$), and cytogenetic poor risk ($p=0.021$). There were no statistically significant differences in age, gender, WBC count, hemoglobin, immunophenotype, WT1, IKZF1, MLL, MRD status, HSCT between two groups.

The median follow-up time was 18 months (range 1–78 months). The CR rates after first cycle of induction therapy were similar between ECM1-Low and ECM1-High group (80.6% vs. 77.1%; $p=0.725$). Kaplan–Meier survival analysis was then performed to compare the outcomes of patients dichotomized by ECM1 expression. Patients with high ECM1 expression had a significantly worse 3-year OS (11.4% vs. 36.1%, $p=0.015$) and 3-year RFS (9.7% vs. 29.4%, $p=0.047$) than those with low ECM1 expression (Fig.2).

High expression of ECM1 at diagnosis independently predicts poor prognosis of adult B-ALL patients

Furthermore, a Cox proportional hazard regression model was set up to assess the effect of ECM1 on the prognosis of adult B-ALL patients. Age ($\geq$ vs. < 35 years), WBC count ($\geq$ vs. $< 30 \times 10^9/L$), BCR-ABL fusion status (negative/positive), treatment (chemotherapy alone vs. chemotherapy/allo-HSCT), MRD at the end of induction therapy (negative/positive), risk group (poor risk vs. standard risk) and ECM1 expression levels (low/high) were included. The univariate cox regression analysis indicated age ≥ 35 years, MRD positive at the end of induction therapy, chemotherapy alone, high ECM1 expression as strong prognostic predictors for poor OS. Besides, MRD positive at the end of induction therapy and chemotherapy alone were also significantly related to poorer RFS. WBC count, BCR-ABL fusion status and risk group had no effects on OS or RFS. To further verify the robustness value of ECM1 expression, multivariate analysis was performed to determine risk assessment related to survival and relapse. Most remarkably, MRD positive at the end of induction therapy, chemotherapy alone and high ECM1 expression were independent adverse prognostic factors for OS. Moreover, MRD positive at the end of induction therapy and chemotherapy alone were independent adverse prognostic factors for RFS. Together, our data revealed that the expression level of ECM1 at diagnosis could serve as an independent prognostic biomarker of clinical outcomes in adult B-ALL patients (Table 3).

Time course analysis of ECM1 expression

To determine the utility of ECM1 as MRD markers, expression of the ECM1 was evaluated at the diagnosis, CR, and relapse. ECM1 and BCR-ABL expression in three continuous CR Ph+ patients during follow-up generally changed in parallel (Fig.3). Expression of the two markers was high at the primary diagnosis and decreased when achieved CR. Furthermore, an increase in expression of ECM1 and BCR-ABL was observed in four relapse Ph+ patients. In addition, among eight continuous CR Ph- patients, decreased expression of ECM1 was observed in four patients who had a high baseline ECM1 expression and achieved CR after induction therapy. Expression of ECM1 was continuous low both at the primary diagnosis and CR for the other four patients. Similarly, an increase in expression of ECM1 was observed in four relapse Ph- patients. In particular, for a patient with negative molecular biomarkers, expression of ECM1 during follow up generally changed in parallel with MRD quantified by analyzing LAIPs using flow

cytometry (Fig.4). Taken together, ECM1 could be a marker of MRD in adult B-ALL patients with negative molecular biomarkers at diagnosis.

Discussion

In the current study, we showed that patients at different state of disease had significantly different ECM1 expression levels, and high expression of ECM1 at diagnosis independently predicated poor outcomes in adult B-ALL.

High ECM1 expression was related to some types of cancers. Moreover, ECM1 was highly and selectively expressed in type 2 helper T cells (TH2) which are critically involved in allergies and asthma¹⁵. ECM1 can regulate M1 macrophage polarization through the granulocyte-macrophage colony-stimulating factor/STAT5 signaling pathway in inflammatory bowel disease (IBD)¹⁶. It has also been reported that ECM1 was statistically significantly elevated in diffuse large B-cell lymphoma patient sera as compared to controls¹⁷. Here we report that ECM1 is also involved in B-ALL. Our results revealed that the expression levels of ECM1 were significantly increased in ND and RR patients than it in CR patients and normal control. Furthermore, among 71 adult patients, high ECM1 expression was independent adverse prognostic factor for OS. Thus, ECM1 might be considered as a novel prognostic indicator in adult patients with B-ALL.

Besides, ECM1-high group has a higher prevalence of B-ALL with high-risk molecular abnormality (BCR-ABL fusion) and more poor risk subtypes, contributing to the poor outcome in patients with high ECM1 expression. However, ECM1-high group also has a higher initial platelet count which was reported as factor prognostic of significantly longer OS¹⁸. This inconsistency may be due to the less prognostic significance of platelet count than BCR-ABL fusion and cytogenetic high risk.

Our data revealed that high expression of ECM1 at diagnosis were associated with both poorer OS and RFS in adult patients with B-ALL. In the multivariate Cox regression model, high ECM1 expression was independently associated with poorer OS, regardless of post-remission therapy, indicating that it was a potential prognostic factor for adult B-ALL. Furthermore, results of the present study confirm MRD positive at the end of induction therapy and chemotherapy alone were independent factors for OS and RFS whereas other variables including age, WBC count, BCR-ABL status and risk group were not. It is well known that MRD is the most powerful prognostic factor in all age groups, including in patients at low risk¹⁹. Our results consistently with previous studies suggested that continuous monitoring of MRD could be useful to detect early preclinical relapse and adaptation of the therapeutic strategy. It is surprising that BCR-ABL status and risk group were not significantly associated with outcomes. There are several possible explanations. First, there were relatively few patients with adverse cytogenetics in addition to Ph chromosome. Second, patients with BCR-ABL received different treatments than patients without BCR-ABL including the combination of TKIs and more prevalence to receive an allotransplant. These interventions might mitigate the adverse impact of BCR-ABL and poor risk.

Besides, to explore the utility of ECM1 as a MRD marker, expression of the ECM1 was evaluated at different disease status of same patients. Reduced expression of ECM1 after induction therapy and increased expression during relapse was observed in whether Ph+ or Ph- patients with follow-up data. Expression of ECM1 during follow up generally changed in parallel with MRD, especially for the patients with negative molecular biomarkers.

Although the results were encouraging, there are still several inadequacies and limitations in our research. First, it was a retrospective study which was susceptible to selection bias. The prognostic value of ECM1 should better be further validated in a validation cohort. Second, several potentially important variables (such as MLL rearrangement, Philadelphia chromosome-like subtype, CNS involvement) were not included in the cox regression analysis because of too few patients or data missing. Third, the functional role of ECM1 in the development of B-ALL remains unknown, which need to be further studied. These potential limitations mean that our conclusion need to be confirmed in a larger, independent prospective cohort and further research on mechanism.

In conclusion, our study demonstrated that ECM1 is over expressed in ND and RR patients in comparison to CR patients and normal control. In adults with B-ALL, high ECM1 expression was independent adverse prognostic factor for OS. Thus, a larger independent prospective cohort of patients with longer-term follow-up is necessary to determine the relevance of ECM1 for the prognosis of adult B-ALL.

Declarations

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Conflicts of interest/Competing interests

Not applicable.

Availability of data and material

Not applicable.

Code availability

Not applicable.

Authors' contributions

Weimin Wang, Zhongxing Jiang, and Feng Guo designed the study; Weimin Wang and Feifei Wu wrote the manuscript; Fei Wang, Congcong Wang, and Chunhui Lyu performed the experiment. Feifei Wu, Lijie Han, Silin Gan, Ping Ma performed statistical analyses and analyzed the data. Zhongxing Jiang and Feng Guo coordinated the study over the entire time. All authors approved the final manuscript.

Ethics approval

The study was approved by the Ethics Committee of the first affiliated hospital of Zhengzhou University. Written informed consent was obtained from adult subjects and from parents of minors in accordance with the Declaration of Helsinki.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Tables

Table 1. Sequences of primers and probes

Name	Sequence (5'-3')
ECM1 forward primer	CCCTACTCGGGATGAGTGCTT
ECM1 reverse primer	TGTAGAACTGGTAACTGTAGCC
ECM1 probe	FAM- TCGGGCTCCTTACCCCA-MGB
ABL forward primer	CCGCTGACCATCAATAAGGAA
ABL reverse primer	GATGTAGTTGCTTGGGACCCA
ABL probe	FAM-CCATTTTTGGTTTGGGCTTCACACCATT-TAMARA

Table 2. Association of ECM1 transcript levels with the clinical characteristics of adult patients with B-ALL

Characteristics	Total	ECM1-Low	ECM-High	<i>p</i> -value
No. of patients n(%)	71	36	35	
Age				
Median(range),year	37(15-72)	35(15-67)	39(17-72)	0.111
≤40 n(%)	40	23(57.5%)	17(42.5%)	0.193
≥40 n(%)	31	13(41.9%)	18(58.9%)	
Gender				0.409
Male	44	24(54.5%)	20(45.5%)	
Female	27	12(44.4%)	15(55.6%)	
WBC count				0.301
Median(range),×10 ⁹ /L	17.5(1.6-586.2)	21.3(1.63-586.2)	9.9(1.86-126.82)	
Hemoglobin,g/L				0.079
Median(range)	82(28.9-158)	78(51-132)	95(55-158)	
Platelet count, ×10⁹/L				0.004
Median(range)	56(4-338)	37(4-210)	84(5-277.7)	
BM blasts,%				0.493
Median(range)	89.0(28.0-98.5)	90.5(28.0-98.5)	86.0(46.0-97.4)	
Immunophenotype				0.291
Pro-B-ALL	10	6(60.0%)	4(40.0%)	
Com-B-ALL	51	23(45.1%)	28(54.9%)	
Pre-B-ALL	10	7(70.0%)	3(30.0%)	
BCR-ABL				0.004
Yes	32	10(31.3%)	22(68.8%)	
No	39	26(66.7%)	13(33.3%)	
WT1				0.924
Yes	43	22(51.2%)	21(48.8%)	
No	28	14(50.0%)	14(50.0%)	
IKZF1				0.072
Yes	26	9(34.6%)	17(65.4%)	

No	14	9(64.3%)	5(35.7%)	
Unknown	31	18	13	
MLL				0.110
Yes	4	4	0	
No	45	21	24	
Unknown	22	11	11	
Risk groups				0.021
Poor risk	41	16(39.0%)	25(61.0%)	
Standard risk	30	20(66.7%)	10(33.3%)	
MRD*				0.557
Positive	24	11	13	
Negative	47	25	22	
HSCT				0.546
Yes	33	18	15	
No	38	18	20	

MRD: a positive MRD-test was defined as cases where $\geq 0.1\%$ of the cells displayed an LAIP phenotype.

Table 3. Predictors of OS and RFS

	Univariate analysis		Multivariate analysis	
	HR*(95%CI*)	<i>p</i> -value	HR (95%CI)	<i>p</i> -value
OS				
Age	2.7(1.38-5.4)	0.004	2.0(0.9-4.3)	0.075
WBC	0.9(0.4-1.6)	0.627	NA*	NA
BCR-ABL	0.7(0.9-3.2)	0.102	1.1(0.4-3.1)	0.923
Risk group	1.6(0.9-3.1)	0.133	2.4(0.8-7.3)	0.129
MRD	3.6(1.9-6.9)	0.000	3.1(1.5-6.4)	0.002
Allo-HSCT	0.3(0.2-0.7)	0.002	0.2(0.1-0.6)	0.002
ECM1	2.6(1.4-4.9)	0.005	3.0(1.4-6.6)	0.007
RFS				
Age	2.4(1.0-5.8)	0.056	1.1(0.4-3.1)	0.895
WBC	0.8(0.4-2.0)	0.704	NA	NA
BCR/ABL	1.0(0.4-2.3)	0.997	NA	NA
Risk group	1.2(0.5-2.7)	0.683	NA	NA
MRD	7.4(3.2-17.6)	0.000	9.1(3.2-25.9)	0.000
Allo-HSCT	0.2(0.1-0.5)	0.001	0.2(0.1-0.5)	0.002
ECM1	1.9(0.8-4.3)	0.145	2.4(1.0-6.0)	0.056

Abbreviations: HR hazard ratio; CI confidence interval; NA not available

Figures

Figure 1

(a): ECM1 transcript levels in four groups. ND represents newly diagnosed patients; CR represents complete remission patients and RR represents refractory and relapse patients. * $p < 0.01$. (b): ECM1 transcript levels in children and adult patients.

Figure 2

Associations between ECM1 expression and RFS/OS in adult B-ALL patients. (a) RFS and (b) OS were compared between patients with low or high ECM1 transcript levels.

Figure 3

ECM1 and BCR-ABL transcript levels correlate with the clinical course of Philadelphia-positive B-ALL patients. (a), (b) and (c) illustrate the levels of ECM1 and BCR-ABL in three continuous CR Ph+ patients during follow-up. (d) and (e) illustrate the levels of ECM1 and BCR-ABL of four patients at the time of ND, CR and relapse respectively.

Figure 4

ECM1 transcript levels correlate with the clinical course of Philadelphia-negative B-ALL patients. (a) illustrates the level of ECM1 in 4 patients at the time of ND and CR. (b) illustrates the level of ECM1 in 4 patients at the time of ND → CR and relapse. (c) illustrates the level of ECM1 in a long-term follow-up patient.