

Expression of Leukocytic Syncytin-1 in B-Cell Acute Lymphoblastic Leukemia and Acute Myeloid Leukemia Patients

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Abstract

Background: Syncytin-1, a cell membrane-localizing fusogen, is improperly expressed in several cancers, including endometrial cancer, breast cancer, and leukemia. Although abnormal syncytin-1 mRNA and protein expression has been detected in two-thirds of leukemia blood samples, its expression profile across leukocytes in acute leukemia patients has not yet been analyzed.

Methods: A total of 50 acute myelogenous leukemia (AML) cases and 14 B-cell acute lymphocytic leukemia (ALL) patients were consecutively recruited. Bone marrow samples were subjected to flow cytometry. Statistical analysis was applied to compare syncytin-1 expression between B-cell ALL and AML across granulocytes, leukemia cells, and T-lymphocytes (including CD3+, CD4+, and CD8+ subsets thereof) and to correlate syncytin-1 expression on leukemia cells and lymphocytes with the T-cell subset percentages.

Results: The percentage of syncytin-1-expressing leukemia cells in AML patients was significantly higher than that in B-cell ALL patients (6.33 ± 6.95 (B-cell ALL) vs. 29.09 ± 27.51 (AML), $P < 0.05$). The percentage of CD8+ T-cells in AML patients was significantly higher than that in B-cell ALL patients (33.34 ± 10.59 (B-cell ALL) vs. 40.00 ± 9.57 (AML), $P < 0.05$). The syncytin-1 expression rate on leukemia cells was positively correlated with the CD8+ T-cell percentage ($r = 0.289$, $P < 0.05$), while the syncytin-1 expression rate on lymphocytes was negatively correlated with the CD3+, CD4+, and CD8+ T-cell percentages ($r = -0.273$, -0.450 , and -0.307 , respectively; $P < 0.05$).

Conclusions: The percentage of syncytin-1-expressing leukemia cells in AML – due to its positive correlation with the CD8+ suppressor T-cell percentage -- shows potential as an indicator of poorer long-term immunity in AML patients.

Keywords: Syncytin; Leukemia, ALL, AML; T-lymphocytes, T-cells

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Introduction

Acute leukemia account for approximately 20,000 diagnoses and 10,000 fatalities in the United States annually [1]. According to the third edition of the International Classification of Diseases for Oncology (ICD-O-3), acute lymphocytic leukemia (ALL, with B-cell and T-cell subtypes) and acute myelogenous leukemia (AML) are the two most common variants of acute leukemia [1]. The pathogenesis of these acute leukemias is a complex process, as inherited genetic factors, viral infection, and radiation exposure have all been associated with the onset of acute leukemias [2]. With respect to viral infection, no specific virus has been

conclusively linked to be a direct cause of acute leukemia [3]. Rather, acute leukemias are more likely to be the result of a dysregulated immune response to common viral infections (such as influenza) in certain susceptible patients [3].

With this in mind, human endogenous retroviruses (HERVs) are believed to be modern remnants of archaic retroviral infections in ancestral germ-line cells [4]. HERV-W is one such HERV with multiple copies present in the human genome that is still capable of being translated into functional proteins [4]. The 538-amino acid residue envelope glycoprotein of HERV-W – termed syncytin-1 – is still expressed in modern humans [5]. In previous functional

studies, syncytin-1 localizes to the cell membrane and effectively functions as a fusogen, as it has been found to be sufficient to induce cell-cell fusion across various cell lines in a receptor-dependent manner [6,7]. Although syncytin-1 has been shown to be expressed in human reproductive tissues including the endometrium, ovary, and testis as well as fusing myoblasts [7,8], syncytin-1 is most notably expressed in placental tissue, where it plays a key role in the fusion of single-nucleated trophoblasts to form a layer of multi-nucleated syncytiotrophoblasts (a structure termed the placental syncytium) [7,9]. This placental syncytium forms a barrier that insulates the fetal circulation from the maternal circulation in order to avoid immune attack from the maternal immune system [7,10]. Accordingly, syncytin-1 expression has been shown to increase from the first to the third trimester [7,11], and the syncytin-1 protein possesses a conserved immunosuppressive domain (amino acid residues 373-397) [12], suggesting that syncytin-1 is involved in suppressing maternal immune responses against the developing fetus. In particular, exosome-mediated syncytin-1 secretion by the placenta to the maternal circulation may play a role in the development of maternal immune tolerance to fetal antigens [7,13].

Similar to placental tissue, exosomes are also produced by lymphocytes, macrophages, and immature dendritic cells, and there are active exchanges of exosomes among these cells during immune responses [7,14]. Moreover, syncytin-1 has been found to be improperly expressed in several human cancers, including endometrial cancer, breast cancer, and leukemia [4]. With respect to leukemia, Sun et al. found abnormal syncytin-1 mRNA and protein expression in more than two-thirds of leukemia blood samples investigated, but neither syncytin-1 transcripts nor protein was detected in blood samples from healthy donors [4]. More recently, Maliniemi et al.'s 2013 study detected syncytin-1 expression in malignant lymphocytes in 50% (15/30) of mycosis fungoides cases under study but found no syncytin-1 expression in inflammatory dermatosis (Lichen ruber planus) with skin-homing, non-malignant T-lymphocytes [15]. These combined findings have led to our research interest on the role of syncytin-1 in malignant immune cells.

Although syncytin-1 expression appears to be associated with leukemia, its expression profile across the various types of leukocytes in leukemia patients has not yet been analyzed. Therefore, this study aims to investigate the expression of syncytin-1 across granulocytes, leukemia cells, and T-lymphocytes (including subsets thereof) in cohorts of B-cell ALL and AML patients.

Materials and Methods

Ethics Statement

This study was approved by the Ethics Committee (IRB) of the First People's Hospital of Yunnan Province. All subjects recruited for this study provided written informed consent prior to participation.

Patient Selection, Sample Collection, and Leukemia Diagnosis

A total of 64 acute leukemia patients were consecutively recruited for this prospective study from the Hematology Clinic of the First People's Hospital of Yunnan Province. No patient was undergoing

treatment for their leukemia at the time of recruitment. From each patient, a bone marrow sample (~0.5 ml) was extracted from the iliac or the anterior superior iliac spine, and a non-fasting peripheral blood sample (2 ml) was drawn into EDTA-K₂ anti-coagulated tubes and stored at room temperature. Using these bone marrow and peripheral blood samples, diagnoses of acute leukemia cases were conducted according to the World Health Organization (WHO) guidelines applying a 20% blast threshold [4,16], and the morphological classification of blast cells was performed according to the French-American-British (FAB) criteria [4,17]. The percentage of malignant leukemia cells for each patient was derived from analysis of bone marrow morphology.

Relative Syncytin-1 mRNA Expression by RT-PCR

Analysis of relative syncytin-1 mRNA expression was performed as previously described with minor modifications [4]. Briefly, MRC TRI Reagent BD was used to extract total RNA from bone marrow samples according to the kit's instruction (Molecular Research Center, Cincinnati, OH, USA). RNA quality was assessed through 1% agarose gel electrophoresis. RNA concentration was determined by 260-nm photoabsorption. Genomic DNA remnants were removed from the RNA samples using a buffered DNase I (#EN0521, Fermentas Life Sciences, Hanover, MD, USA). Then, real-time RT-PCR was performed using a One Step SYBR RT-PCR Kit in an ABI 7000 (Applied Biosystems, USA) according to the kit's instruction (TaKaRa Biotechnology Co., Ltd., Dalian, China). Three reactions were run in parallel for each sample: (i) a RT-PCR reaction with 0.75 µg DNase I-treated RNA template + reverse transcriptase, (ii) a control lacking the RNA template, and (iii) a control lacking reverse transcriptase. The syncytin-1 (gi.21326140) and GADPH (gi.182860) primers used for RT-PCR amplification have been described previously with the expected PCR products of 494 bp (syncytin-1) and 358 bp (GADPH) [4,18]. The RT-PCR reaction was run in the following sequence: 42°C for 15 min, then 95°C for 2 min followed by 35 cycles of 95°C for 5 s and 60°C for 34 s. Dissociation curves and agarose gel electrophoresis were used to analyze RT-PCR amplification.

Relative mRNA expression of syncytin-1 (relative to mRNA expression of the internal control GADPH) were analyzed by the $2^{-\Delta\Delta Ct}$ method [4,19]. As previously described by Sun et al., total RNA extracted from C8166 cells (Kunming Institute of Zoology, Chinese Academy of Sciences) was used as a reference in calculating syncytin-1 mRNA expression levels [4]. These C8166 cells were maintained in RPMI-1640 medium supplemented with 10% heat inactivated fetal calf serum (Gibco), 100 IU/mL penicillin, and 100 µg/mL streptomycin at 37°C in a humidified 5% CO₂ incubator and then harvested at mid-log phase [4].

Flow Cytometry

The flow cytometric procedure was conducted as follows. Briefly, EDTA-anticoagulated bone marrow aspirates were stained for flow cytometric analysis within 24 hours of specimen collection. Control tubes were prepared as follows: 10 µl phycoerythrin-Cy5-conjugated anti-human CD45 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was added to PBS-diluted bone marrow cells (100 µl of 4-10 × 10⁹ cells/l), gently shaken, and then incubated in

the dark for 20 minutes after adding a hemolytic 1 × ammonium chloride (NH₄Cl) lysing solution. In the dark, washing liquid was used to completely rinse off the hemolytic agent, and PBS was added prior to flow cytometric testing.

Non-control test tubes were prepared with PBS-diluted bone marrow cells (100 μl of 4-10 × 10⁹ cells/l) and the following fluorescent antibodies (10 μl each): rabbit anti-human syncytin-1 (H-280, Santa Cruz Biotechnology, Santa Cruz, CA, USA), phycoerythrin-Cy5-conjugated anti-human CD45, phycoerythrin-Cy5-conjugated anti-human CD3, phycoerythrin-conjugated anti-human CD4, and ECD-conjugated anti-human CD8 (Beckman-Coulter, USA). Tubes were gently shaken and then incubated in the dark for 20 minutes after adding a hemolytic 1 × ammonium chloride (NH₄Cl) lysing solution. Then, washing liquid was used to completely rinse off the hemolytic agent. Then, 10 μl FITC-conjugated goat anti-rabbit IgG (KPL, Gaithersburg, MD, USA) was added and then incubated in the dark for 20 minutes. Each sample was then washed and resuspended in 1.5 ml PBS prior to flow cytometric testing.

Multiple-color flow cytometry was performed using a FC500 flow cytometer (Beckman-Coulter, USA). Amplification and compensation were performed according to standard procedures. Flow cytometry was performed with two test tubes: a negative control and an isotype control. For the negative control, 10 μl CD45 was added to a matching amount of bone marrow cells and incubated in the dark for 15-20 minutes. A hemolytic agent was added to the sample until the liquid cleared and washed with PBS prior to flow cytometry. For the isotype control, 10 μl CD45 and 10 μl monoclonal anti-syncytin-1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was added to a matching amount of bone marrow cells and incubated in the dark for 15-20 minutes. A hemolytic agent was added to the sample until the liquid cleared and washed with PBS. Then, 10 μl FITC-labeled goat anti-rabbit IgG (KPL, USA) were added for incubation in the dark for 15-20 minutes. The sample was then washed with PBS prior to flow cytometry. At least 10000 cell events were collected for all samples. CD45 versus side scatter was used to select four populations of cells: granulocytes, monocytes, lymphocytes, and malignant leukemia cells. Multiparameter analysis of antibody-staining patterns was applied to analyze the various cell populations for differing trends in surface antigen expression. Test results were expressed in percentages.

Statistical Analysis

The statistical analysis was performed with SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). The Wilcoxon rank sum test was used to compare syncytin-1 expression between B-cell ALL and AML across granulocytes and T-lymphocytes (including CD3+, CD4+, and CD8+ subsets thereof), as the data did not follow a normal distribution according to one-sample Kolmogorov-Smirnov normality testing. All other comparisons were conducted by independent two-sample *t*-testing, as the one-sample Kolmogorov-Smirnov normality test revealed normal distributions for these other comparisons. For correlation analyses, a Spearman's correlation was used to correlate syncytin-1 expression on leukemia cells with CD3+, CD4+, and CD8+ T-cell subset percentages (as this was a non-normally-distributed dataset), while a Pearson's correlation

was used to correlate syncytin-1 expression on lymphocytes with CD3+, CD4+, and CD8+ T-cell subset percentages (as this was a normally-distributed dataset).

Results

The characteristics of the 64 recruited leukemia patients are detailed in **Table 1**. The mean and median age of these patients were 38.2 and 37, respectively, with an age range of 4-78. There were 40 males (62.5%) and 24 females (37.5%) in the cohort. There were 50 cases of AML (78.1%) and 14 cases of B-cell ALL (21.9%). The mean and median WBC count for the cohort were 34.6 and 14.9 (×10⁹/l), respectively, with a WBC count range of 0.96-293.7 (×10⁹/l). The mean and median percentage of malignant cells in each sample for the cohort were 62.6% and 72.4%, respectively, with a malignant cell percentage range of 0.41-98.5%.

With respect to syncytin-1 mRNA expression in bone marrow cells from both cohorts, we detected a significant difference in syncytin-1 mRNA expression from B-cell ALL patients and AML patients after conducting two independent sample *t*-testing (*P*=0.033). By Spearman correlation analysis, we found no significant correlations between syncytin-1 mRNA expression and the percentage of syncytin-1-expressing granulocytes, the percentage of syncytin-1-expressing leukemia cells, or the percentage of syncytin-1-expressing lymphocytes (or any of the subsets thereof) (all *P*>0.05).

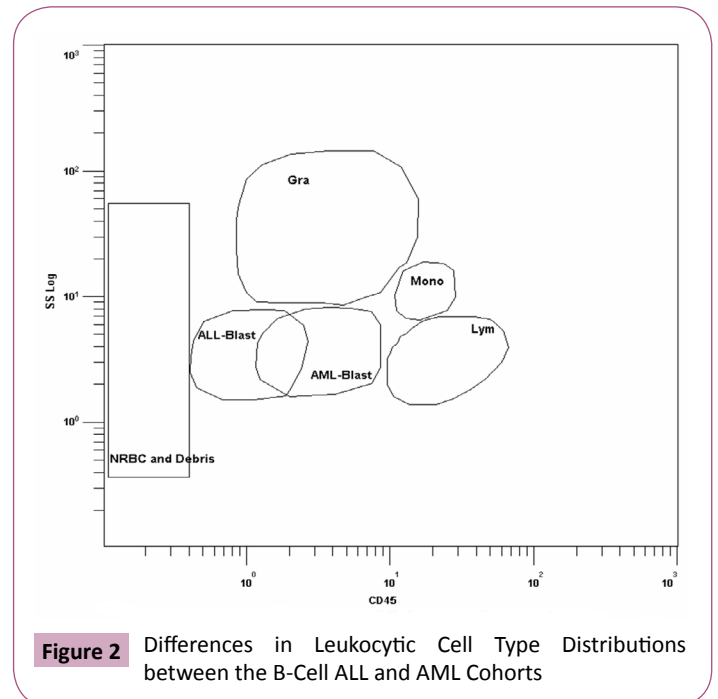
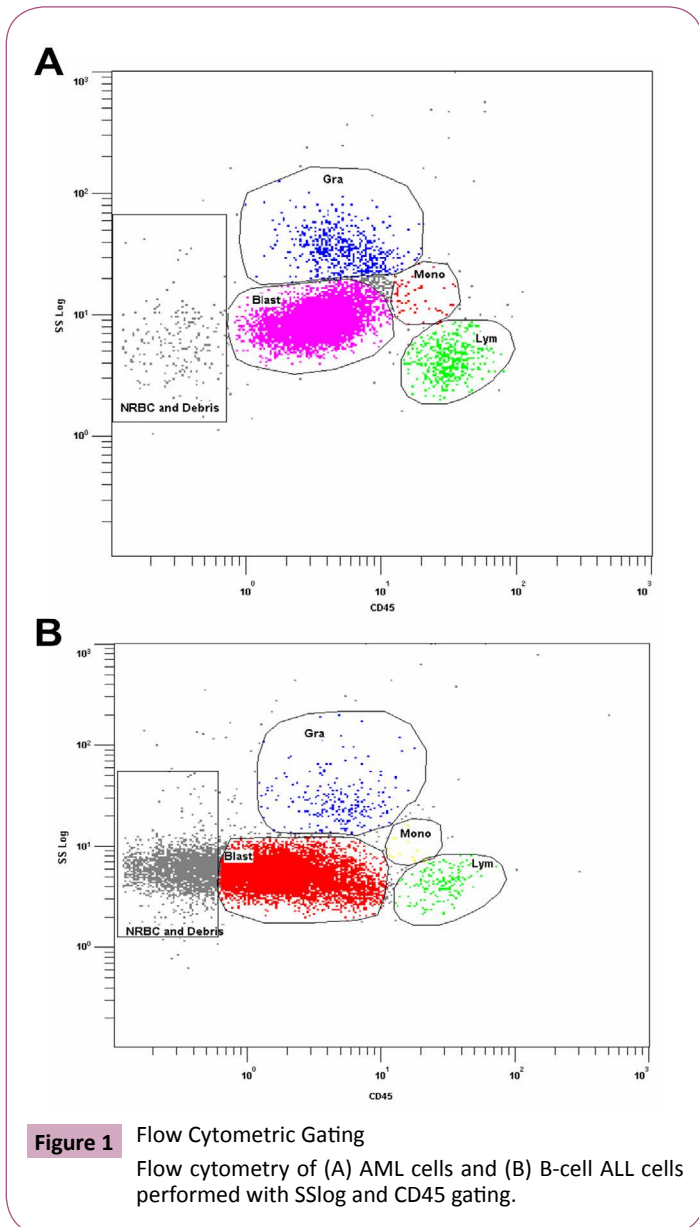
Flow cytometry figures depicting CD45 gating have been provided in **Figure 1**, and the differences in leukocytic cell type distributions between the B-cell ALL and AML cohorts have been provided in **Figure 2**. Using flow cytometry, we compared syncytin-1 expression across granulocytes, leukemia cells, and T-lymphocytes (including subsets thereof) between the B-cell ALL and AML cohorts (**Table 2**). The percentage of syncytin-1-expressing leukemia cells in AML patients was significantly higher than that in B-cell ALL patients (6.33 ± 6.95 (B-cell ALL) vs. 29.09 ± 27.51 (AML), *P*<0.05; **Table 2**). However, the percentages of syncytin-1-expressing granulocytes (45.39 ± 27.48 (B-cell ALL) vs. 40.95 ± 23.08 (AML), *P*>0.05) and syncytin-1-expressing lymphocytes (20.79 ± 10.22 (B-cell ALL) vs. 23.79 ± 13.68 (AML), *P*>0.05) were not significantly different between these two patient populations (**Table 2**). Moreover, the percentage of CD8+ T-cells in AML patients was significantly higher than that in B-cell ALL patients (33.34 ± 10.59 (B-cell ALL) vs. 40.00 ± 9.57 (AML), *P*<0.05; **Table 2**). However, the percentages of CD3+ T-cells (79.59 ± 10.27 (B-cell ALL) vs. 78.15 ± 10.55 (AML), *P*>0.05) and CD4+ T-cells (42.34 ± 12.13 (B-cell ALL) vs. 36.56 ± 9.98 (AML)), *P*>0.05) were not significantly different between these two patient populations (**Table 2**). As a result, the CD4+/CD8+ ratio was significantly lower in AML patients than in B-cell ALL patients (1.44 ± 0.74 (B-cell ALL) vs. 0.98 ± 0.39 (AML), *P*<0.05;) (**Table 2 and Figure 3**).

Finally, we correlated the CD3+, CD4+, and CD8+ T-cell subset percentages with syncytin-1 expression rates on leukemia cells and lymphocytes (**Table 3**). The syncytin-1 expression rate on leukemia cells was positively correlated with the percentage of CD8+ T-cells (*r*=0.289, *P*<0.05; **Table 3 and Figure 4**). The syncytin-1 expression rate on lymphocytes was negatively correlated with the percentages of CD3+, CD4+, and CD8+ T-cells

Table 1 Register of patient characteristics.

Age (yrs)	Sex	Leukemia diagnosis	WBC count (10 ⁹ /l)	Percentage of malignant cells in bone marrow (%)	Relative syncytin-1 mRNA expression (syncytin-1/GAPDH, means ± SD)
B-cell Acute Lymphoblastic Leukemia (B-cell ALL) Patients (n=14)					
12	M	B-cell ALL	5.5	9.5	9.3 ± 0.2
22	F	B-cell ALL	2.16	98.5	0.5 ± 0.3
67	M	B-cell ALL	5.75	74	4 ± 1.9
22	M	B-cell ALL	9.73	68.5	3.7 ± 0.4
23	M	B-cell ALL	4.8	92.5	19.1 ± 8.1
18	M	B-cell ALL	2.36	75	10 ± 2.8
6	F	B-cell ALL	4.38	84.5	11.2 ± 0.8
17	F	B-cell ALL	4.5	0.41	0.17 ± 0.1
50	M	B-cell ALL	42.7	96	0.32 ± 0.2
25	M	B-cell ALL	4.54	83.5	0.99 ± 0.4
46	M	CML→B-cell ALL	51.5	89.5	1.2 ± 0.5
39	F	CML→B-cell ALL	16.8	80.5	3.7 ± 0.5
41	M	CML→B-cell ALL	13	74.5	3.1 ± 0.6
46	F	CML→B-cell ALL	293.7	67.8	21.4 ± 9.2
Acute Myeloid Leukemia (AML) Patients (n=50)					
4	M	M0 AML	45.9	39	13.3 ± 6.9
49	M	M1 AML	3.19	3	3.7 ± 1.2
51	M	M2 AML	10.4	52	33.7 ± 6.5
52	M	M2 AML	20.2	0.8	21.6 ± 3.3
17	F	M2 AML	11.5	24.5	17.4 ± 6.4
64	F	M2 AML	9.78	10.6	11.4 ± 7.7
43	M	M2 AML	11.2	18	11.8 ± 2.7
54	M	M2 AML	6.12	45	1.11 ± 2.5
23	F	M3 AML	18	93.5	27.7 ± 10.9
30	M	M3 AML	45.8	96	0.6 ± 0.2
72	M	M3 AML	0.96	65	27.3 ± 12.9
78	F	M3 AML	124	93.5	2.3 ± 0.4
46	F	M3 AML	1.6	45	29.8 ± 7.4
33	M	M3 AML	20.2	80.4	37.8 ± 5.5
35	F	M3 AML	28.1	95	15.7 ± 7.8
18	F	M3 AML	1.17	76.5	22 ± 4.7
37	M	M3 AML	10.4	52	22.1 ± 8.2
78	M	M3 AML	23.5	94	16.1 ± 7.9
35	F	M3 AML	11	3	15.1 ± 4.9
32	M	M3 AML	82.2	77	15.8 ± 8.3
17	M	M3 AML	11.9	72.7	39.6 ± 10.6
56	M	M3 AML	33.5	89.5	29.4 ± 5.6
37	M	M4 AML	4.33	39.5	4.2 ± 2.7
53	M	M4 AML	1.75	66.5	76.3 ± 0.9
48	F	M4 AML	22.3	72	7 ± 2.7
37	F	M4 AML	2.73	59.5	83.7 ± 24.7
12	F	M4 AML	2.02	64.3	27 ± 11.0
25	M	M4 AML	292	84	2.5 ± 0.8
39	F	M4 AML	17.3	30.12	21.1 ± 6.4
42	M	M4 AML	29.8	76.5	7.9 ± 0.9
25	M	M4 AML	7.86	34	0.37 ± 0.2
48	M	M5 AML	4.81	96.7	66.3 ± 9.8
16	M	M5 AML	43.7	91.5	82.2 ± 13.5
13	F	M5 AML	86.6	91.5	2.4 ± 0.6

54	F	M5 AML	18.5	68	63.7 ± 12.9
61	F	M5 AML	23.7	89	16.9 ± 2.3
59	M	M5 AML	3.44	93.5	18.8 ± 6.4
46	M	M5a AML	75.7	54.2	77.8 ± 15.5
44	F	M5a AML	28.1	27.8	83.6 ± 12.8
29	F	M5a AML	18.5	45	53.7 ± 16.6
36	M	M5b AML	47.9	94.5	81.2 ± 10.5
58	F	M5b AML	95.9	81	76.8 ± 20.3
18	M	M5b AML	163	85	87 ± 12.9
37	F	M5b AML	25.6	89.5	34.6 ± 6.6
72	M	M5b AML	148	87.5	37.6 ± 13.7
28	M	M6 AML	27.5	51.8	3.8 ± 1.3
20	M	CML→AML	1.71	34	1 ± 0.7
29	M	CML→AML	25.5	20.5	3.7 ± 1.7
27	M	CML→M5 AML	2.54	57.5	5.87 ± 1.3
72	M	AML (NOS)	2.83	3.5	12.3 ± 2.8



($r=-0.273$, -0.450 , and -0.307 , respectively; $P<0.05$; **Table 3** and **Figure 5**).

Discussion

Based on the classical model of hematopoietic commitment and blood lineage development, hematopoietic stem cells (HSCs) can differentiate along one of two cell lineages: a myeloid lineage (which produces granulocytes, monocytes, erythrocytes, and platelets) or a lymphoid lineage (which produces B-cells and T-cells) [20-22]. Malignant transformation in myeloid lineage progenitors produce AML cells, while malignant transformation in lymphoid lineage progenitors produce ALL cells. Here, using flow cytometry, we investigated syncytin-1 expression across granulocytes, leukemia cells, and T-lymphocytes (including subsets thereof) in cohorts of B-cell ALL and AML patients. We found that the percentage of syncytin-1-expressing leukemia cells in AML patients was significantly higher than that in B-cell

ALL patients, but the percentages of syncytin-1-expressing granulocyte and syncytin-1-expressing lymphocytes were not significantly different between these two patient populations (Table 2). Therefore, the current findings suggest syncytin-1 expression may play a role in the development and maintenance of the AML cell phenotype but not the B-cell ALL cell phenotype.

Table 2 Differences in leukocytic cell type distributions between B-Cell acute lymphoblastic leukemia and acute myeloid leukemia patients.

Cell type	B-cell ALL patients (n=14)	AML patients (n=50)	T-value	P-value
Syncytin-1-expressing granulocyte %	45.39 ± 27.48	40.95 ± 23.08	0.565	0.574
Syncytin-1-expressing leukemia cell %*	6.33 ± 6.95	29.09 ± 27.51	27.005 (chi-square)	<0.001
Syncytin-1-expressing lymphocyte %	20.79 ± 10.22	23.79 ± 13.68	-0.763	0.449
CD3+ T-cell %	79.59 ± 10.27	78.15 ± 10.55	0.447	0.657
CD4+ T-cell %	42.34 ± 12.13	36.56 ± 9.98	1.781	0.080
CD8+ T-cell %*	33.34 ± 10.59	40.00 ± 9.57	-2.209	0.031
CD4/CD8 ratio*	1.44 ± 0.74	0.98 ± 0.39	2.204	0.043

In addition, we found that the percentage of CD8+ suppressor T-cells in AML patients was significantly higher than that in B-cell ALL patients, and the percentage of CD8+ suppressor T-cells was positively correlated with syncytin-1 expression rate on leukemia cells ($r=0.289, P<0.05$; Table 3 and Figure 4). However, the percentages of CD3+ and CD4+ helper T-cells were not significantly different between AML and B-cell ALL cohorts (Table 2). As a result, the CD4/CD8 (helper/suppressor) T-cell ratio was significantly low in AML patients than in B-cell ALL patients (Table 2). These combined findings suggest that the heightened syncytin-1 expression on AML cells may preferentially promote the CD8+ suppressor T-cell percentage with accompanying decreases in effector- or effector-memory-cells and less central-memory-cells [23,24]. Or, conversely, these findings can also indicate that the heightened CD8+ suppressor T-cell percentage observed in AML patients promotes syncytin-1 expression in AML cells. Regardless of causation, the percentage of syncytin-1-expressing leukemia cells in AML – due to its positive correlation with the CD8+ suppressor T-cell percentage -- shows potential as an indicator of poorer long-term immunity in AML patients [23-25]. Thus, future clinical studies should focus on the relationship (if any) between syncytin-1 expression in AML cells, high-risk prognostic features (e.g., hepatomegaly/splenomegaly, elevated serum lactic dehydrogenase level, presence of a mediastinal mass [25]), and the long-term clinical outcomes of AML patients.

Mechanistically, the positive relationship between syncytin-1 expression in AML cells and the CD8+ suppressor T-cell percentage

Table 3 Correlation analysis of syncytin expression on the T-Cell subpopulations.

Syncytin-1 expression (%)		CD3+ T-cell %	CD4+ T-cell %	CD8+ T-cell %	CD4+/CD8+ ratio
Syncytin-1 expression on leukaemia cells (%)	r	-0.011	-0.147	0.289*	-0.255
	P	0.936	0.275	0.029	0.056
Syncytin-1 expression on lymphocytes (%)	r	-0.273*	-0.450*	0.158	-0.307*
	P	0.040	<0.001	0.240	0.020

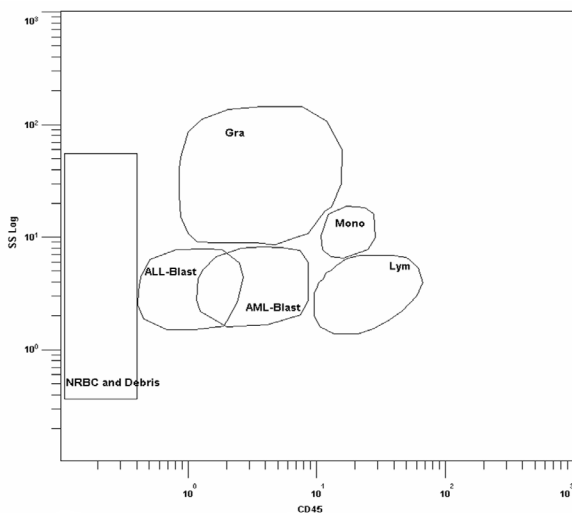


Figure 3 Leukocytic Syncytin-1 Expression in the B-Cell ALL and AML Cohorts

Syncytin-1 expression (as expressed in relative fluorescence units (RFU)) across all leukocytes (i.e., granulocytes, leukemia cells, and T-lymphocytes (including subsets thereof) in the B-cell ALL and AML cohorts. The AML cohort displayed significantly higher leukocytic syncytin-1 expression over the B-cell ALL cohort (** $P=0.001$).

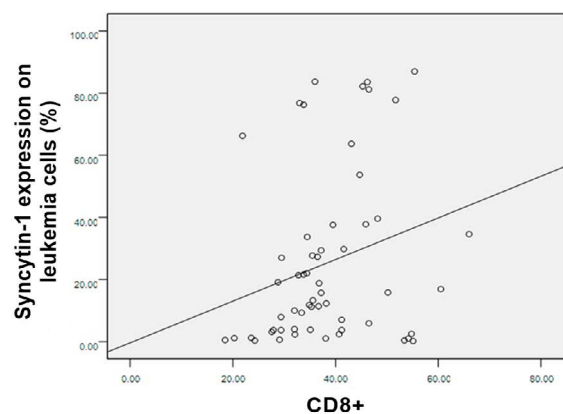


Figure 4 Correlation Analysis of Syncytin-1 Expression on Leukemia Cells

The syncytin-1 expression rate on leukemia cells was positively correlated with the percentage of CD8+ T-cells ($r = 0.289, P<0.05$).

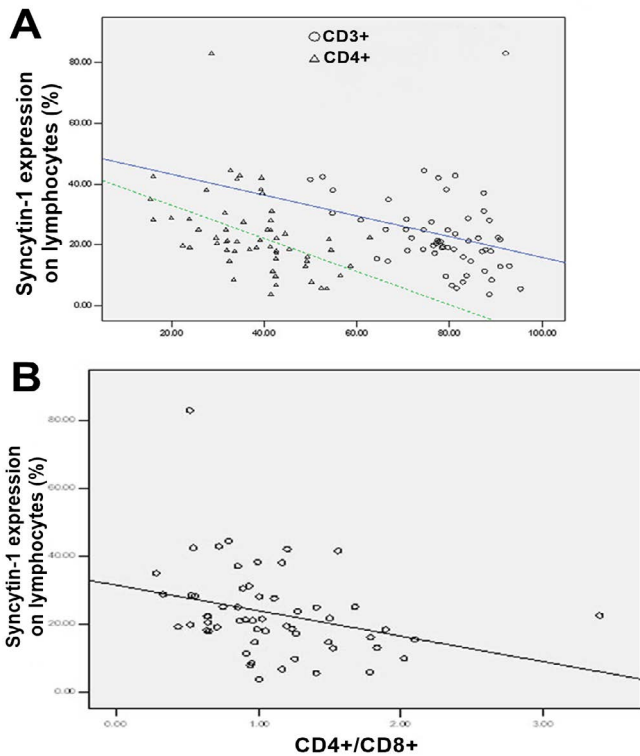


Figure 5 Correlation Analysis of Syncytin-1 Expression on the T-Cell Subpopulations

(A) The syncytin-1 expression rate on lymphocytes correlated with the percentages of CD3+ and CD4+ T-cells. (B) The syncytin-1 expression rate on lymphocytes correlated with the CD4+/CD8+ ratio. The syncytin-1 expression rate on lymphocytes was negatively correlated with the percentages of CD3+, CD4+, and CD8+ T-cells ($r = -0.273$, -0.450 , and -0.307 , respectively; $P < 0.05$).

may be associated with syncytin-1's immunosuppressive properties. Although little is known about syncytin-1's role in immunity, it is noteworthy that syncytin-1 and HIV-1's gp160 – both of which are envelope proteins with immunosuppressive properties -- share several common structural features. First, the syncytin-1 precursor 73-Kd gPr73 is cleaved into a 50-Kd surface subunit (SU) and a 24-Kd transmembrane subunit (TM), while HIV-1 gp160 is cleaved into gp120 (a surface subunit) and gp41 (a

transmembrane subunit) [4,5,26]. Second, structural analysis of syncytin-1 has revealed an immunosuppressive peptide domain (amino acid residues 373-397), while the HIV-1 gp41 peptide also has a 17-amino acid segment with similar immunosuppressive functions [4,12,26]. On this basis, similar to the aforementioned role of syncytin-1 in the placenta [7,13], we speculate that heightened exosome-mediated syncytin-1 secretion on the cell surface of transformed AML cells may play a role in the development of immune tolerance. Moreover, on account of syncytin-1's fusion activity [7,9], heightened syncytin-1 secretion may also be conducive to improved AML cell migration.

Conclusion

We found that the percentage of syncytin-1-expressing leukemia cells in AML patients was significantly higher than that in B-cell ALL patients. Moreover, the percentage of CD8+ suppressor T-cells in AML patients was significantly higher than that in B-cell ALL patients, and the percentage of CD8+ suppressor T-cells was positively correlated with the syncytin-1 expression rate on leukemia cells. Therefore, the percentage of syncytin-1-expressing leukemia cells in AML – due to its positive correlation with the CD8+ suppressor T-cell percentage -- shows potential as an indicator of poorer long-term immunity in AML patients [23-25]. Although this study has analyzed the relationship between syncytin-1 expression and CD3+, CD4+, and CD8+ T-cell subsets in AML and B-cell ALL, the relationship between syncytin-1 expression and other lymphocyte subsets in other leukemic conditions requires further study. In addition, future clinical studies should focus on the relationship (if any) between syncytin-1 expression in AML cells, high-risk prognostic features, and the long-term clinical outcomes of AML patients.

Supplementary Information

Supplementary Figure 1 Scatterplots and Raw Data from the Flow Cytometric Experiments

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