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ORIGINAL ARTICLE

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

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SUMMARY

Background: **Syncytin-1 is improperly expressed in several cancers. However, its expression profile across leukocytes in leukemia patients has not yet been analyzed.**
Methods: **A total of 50 AML cases and 14 B-cell 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 to leukemia cells and lymphocytes with the T-cell subset percentages.**
Results: **The syncytin-1-expressing leukemia cell% in AML patients was significantly higher than that in B-cell ALL patients ($p < 0.05$). The CD8+ T-cell% in AML patients was significantly higher than that in B-cell ALL patients ($p < 0.05$). 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.**
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INTRODUCTION

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]. In the United States, the annual incidence of ALL and AML was 1.8 and 4.3, respectively, per 100,000 men and women in 2014 [2]. In China, no systematic epidemiological data is available so far. It is reported that leukemia is one of the ten leading causes of cancer death [3]. The average yearly incidence rate of leukemia was

3.68 per 100,000 from 2003 to 2007 in Nanjing, which is the second largest city in southeast China [4]. The pathogenesis of these acute leukemias is a complex process, as inherited genetic factors, exogenous (e.g., radiation) and endogenous (e.g., inflammation, oxidative stress) exposures, have all been associated with the onset of acute leukemias [5]. Viral infection was a candidate of causal exposure for childhood acute leukemia, which was suggested by some epidemiological data. But it remains hypothetical due to lack of enough evidence for biological mechanisms of infectious promotion of acute leukemia [6].

With this in mind, human endogenous retroviruses (HERVs) are believed to be modern remnants of archaic retroviral infections in ancestral germ-line cells [7]. HERV-W is one such HERV with multiple copies present in the human genome that are still capable of being translated into functional proteins [7]. The 538-amino acid residue envelope glycoprotein of HERV-W - termed syncytin-1 - is still expressed in modern humans [8]. 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 [9,10]. Although syncytin-1 has been shown to be expressed in human reproductive tissues including the endometrium, ovary, and testis as well as fusing myoblasts [10,11], 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) [10,12]. 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 [10,13]. Accordingly, syncytin-1 expression has been shown to increase from the first to the third trimester [10,14], and the syncytin-1 protein possesses a conserved immunosuppressive domain (amino acid residues 373 - 397) [15], 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 [10,16]. 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 [10,17]. Moreover, syncytin-1 has been found to be improperly expressed in several human cancers, including endometrial cancer, breast cancer, and leukemia [7]. 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 [7]. 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 [18]. 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

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-K_2 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 [7, 19], and the morphological classification of blast cells was performed according to the French-American-British (FAB) criteria [7,20]. 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 [7]. 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 am-

plification 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 minutes, then 95°C for 2 minutes followed by 35 cycles of 95°C for 5 seconds and 60°C for 34 seconds. 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 previously described by Stetler-Stevenson et al. with minor modifications [23]. Briefly, heparinized 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 x 10^9 cells/L), gently shaken, and then incubated in the dark for 20 minutes after adding a hemolytic 1 x ammonium chloride (NH_4Cl) 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 x 10^9 cells/L) and the following fluorescent antibodies (10 μL each): monoclonal anti-human syncytin-1, phycoerythrin-Cy5-conjugated anti-human CD45, phycoerythrin-Cy5-conjugated anti-human CD3, phycoerythrin-conjugated anti-human CD4, and ECD-conjugated anti-human CD8 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Tubes were gently shaken and then incubated in the dark for 20 minutes after adding a hemolytic 1 x ammonium chloride (NH_4Cl) 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. After washing with PBS, PBS was added 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. For each tube, a corresponding isotype control was prepared by incubating the cells with the same volume or dilution of the same approximate secondary antibody of FITC-conjugated isotype IgG, and a negative control was processed as described above except that the primary antibody was excluded. At least 10,000 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. Additional details regarding the flow cytometry instrumentation, gating strategy and data analysis are provided in Supplementary Table 1.

Statistical analysis

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, 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 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). Factors affecting complete remission (CR) probability were studied by univariate analysis and multivariate logistic regression.

RESULTS

The characteristics of the 64 recruited leukemia patients are detailed in Table 1. The mean and median age for 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 (x 10^9/L), respectively, with a WBC count range of 0.96 - 293.7 (x 10^9/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%. Using flow cytometry (Figure 1), 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 percent-

Table 1. Register of patient characteristics.

Age (yrs)	Gender	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

Table 1. Register of patient characteristics (continue).

Age (yrs)	Gender	Leukemia diagnosis	WBC count (10^9/L)	Percentage of malignant cells in bone marrow (%)	Relative syncytin-1 mRNA expression (syncytin-1/GAPDH, means ± SD)
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

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

* - $p < 0.05$.

Table 3. Syncytin-1 expression in leukocytic cell type distributions in FAB subtypes of AML.

Cell type	M0	M1	M2	M3	M4	M5	M6
Syncytin-1-expressing granulocyte %	34.89 ± 0	33.12 ± 0	42.36 ± 23.31	46.53 ± 26.56	54.43 ± 30.26	48.34 ± 26.98	32.46 ± 0
Syncytin-1-expressing leukemia cell %	25.66 ± 0	17.86 ± 0	29.41 ± 19.58	37.43 ± 26.53	39.75 ± 28.43	45.56 ± 31.77	18.21 ± 0
Syncytin-1-expressing lymphocyte %	18.82 ± 0	19.56 ± 0	25.65 ± 13.43	28.43 ± 16.67	31.67 ± 18.11	28.43 ± 14.21	18.90 ± 0

Table 4. Correlation analysis of Syncytin-1 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 leukemia 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

* - $p < 0.05$.

Table 5. Uni- and multivariate analysis of potential factors for achievement of complete remission for AML patients.

Variable	Multivariate	
	Relative risk (95% confidence interval)	p
FAB subtype	0.782 (0.643 - 0.814)	0.026
Age	0.703 (0.552 - 0.895)	0.034
Gender	0.925 (0.731 - 1.162)	0.492
WBC count	1.024 (0.765 - 1.375)	0.873
Percentage of malignant cells in bone marrow	0.782 (0.643 - 0.814)	0.026
Syncytin-1-expressing leukemia cell	0.615 (0.522 - 0.735)	0.014

ages 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 3). 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). Specifically, syncytin-1 expression in Leukocytic Cell Type Distributions in FAB subtypes of AML has been evaluated further, but no significant difference was shown among groups (Table 3).

We correlated the CD3+, CD4+, and CD8+ T-cell subset percentages with syncytin-1 expression rates on leukemia cells and lymphocytes. 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 4). 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; $p < 0.05$; Table 4). Finally, the role of syncytin-1 expression in the outcome of AML patients was studied by univariate analysis and multivariate logistic regression. CR after induction therapy was achieved in 35 AML patients (70%): syncytin-1-expressing leukemia cells in the CR group were significantly lower compared to the non-CR group. In univariate analysis, other factors positively affecting CR included: FAB subtype, age, WBC count, and percentage of malignant cells in bone marrow. In multivariate logistic regression, statistical significance was retained by syncytin-1-expressing leukemia cells, age, FAB subtype, and percentage of malignant cells in bone marrow (Table 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) [24,25]. Malignant transformation in myeloid lineage progenitors produce AML cells, while malignant transformation in lymphoid linage 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. 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). However, the percentages of CD3+ and CD4+ helper T-cells were not significantly different between AML and B-cell ALL (Table 2). As a result, the CD4/CD8 (helper/suppressor) T-cell ratio was significantly lower 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 [26,27]. 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 [26-28]. However, the r 0.289 suggests CD8 suppressor cells with syncytin-1 expression is a weak correlation. It may be interpreted that CD8 suppressor cells and syncytin-1 expression are indirectly connected or concerned with other factors. By univariate analysis and multivariate logistic regression, we found syncytin-1 expression in addition to some other factors affect CR probability in AML patients, which suggests its vital role in AML development and potential as an indicator for AML poor prognosis. 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 [28]).

Mechanistically, the positive relationship between syncytin-1 expression in AML cells and the CD8+ suppressor T-cell percentage 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) [7,8,29]. 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 [7,15,29]. On this basis, similar to the aforementioned role of syncytin-1 in the placenta [10,16], 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 [10,12], 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 [26-28]. 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.

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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.

Declaration of Interest:
None.

References:

1. Dores GM, Devesa SS, Curtis RE, Linet MS, Morton LM. Acute leukemia incidence and patient survival among children and adults in the United States, 2001-2007. Blood 2012;119(1):34-43 (PMID: 22086414).

2. Howlader N, Noone AM, Krapcho M: SEER Cancer Statistics Review (CSR) 1975-2014. Bethesda, MD: National Cancer Institute, Available at URL: https://seer.cancer.gov/csr/1975_2014/

3. Chen W, Zheng R, Zeng H, et al. The incidence and mortality of major cancers in China, 2012. Chin J Cancer 2016,35(1):73 (PMID: 27484217).

4. Chen BA, Huang ZH, Zhang XP, et al. An epidemiological investigation of leukemia incidence between 2003 and 2007 in Nanjing, China. J Hematol Oncol 2010,3:21 (PMID: 20515504).

5. Greaves M. Aetiology of acute leukaemia. Lancet 1997;349 (9048):344-9 (PMID: 9024390).

6. Inaba H, Greaves M, Mullighan CG. Acute lymphoblastic leukaemia. Lancet 2013;381(9881):1943-55 (PMID: 23523389).

7. Sun Y, Ouyang DY, Pang W, et al. Expression of syncytin in leukemia and lymphoma cells. Leuk Res 2010;34(9):1195-202. (PMID: 20362331).

8. Gong R, Peng X, Kang S, et al. Structural characterization of the fusion core in syncytin, envelope protein of human endogenous retrovirus family W. Biochem Biophys Res Commun 2005;331 (4):1193-200 (PMID: 15883002).

9. Aguilar PS, Baylies MK, Fleissner A, et al. Genetic basis of cell-cell fusion mechanisms. Trends Genet 2013;29(7):427-37 (PMID: 23453622).

10. Huang Q, Chen H, Li J, et al. Epigenetic and non-epigenetic regulation of syncytin-1 expression in human placenta and cancer tissues. Cell Signal 2014;26(3):648-56 (PMID: 24216608).

11. Bjerregaard B, Talts JF, Larsson LI. The endogenous envelope protein syncytin is involved in myoblast fusion. Cell Fusions: Springer; 2011. p. 267-75. http://link.springer.com/chapter/10.1007/978-90-481-9772-9_13?no-access=true

12. Huppertz B, Borges M. Placenta trophoblast fusion. Methods Mol Biol 2008;475:135-47 (PMID: 18979242).

13. Erlebacher A. Immunology of the maternal-fetal interface. Annu Rev Immunol 2013;31:387-411 (PMID: 23298207).

14. Chen CP, Wang KG, Chen CY, Yu C, Chuang HC, Chen H. Altered placental syncytin and its receptor ASCT2 expression in placental development and pre-eclampsia. BJOG 2006;113(2): 152-8 (PMID: 16411991).

15. Mi S, Lee X, Li XP, et al. Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. Nature 2000;403(6771):785-9 (PMID: 10693809).

16. Tolosa JM, Schjenken JE, Clifton VL, et al. The endogenous retroviral envelope protein syncytin-1 inhibits LPS/PHA-stimulated cytokine responses in human blood and is sorted into placental exosomes. Placenta 2012;33(11):933-41 (PMID: 22999499).

17. Bobrie A, Colombo M, Raposo G, Théry C. Exosome secretion: molecular mechanisms and roles in immune responses. Traffic 2011;12(12):1659-68 (PMID: 21645191).

18. Maliniemi P, Vincendeau M, Mayer J, et al. Expression of Human Endogenous Retrovirus-W Including Syncytin-1 in Cutaneous T-Cell Lymphoma. PloS one 2013;8(10):e76281 (PMID: 24098463).

19. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. Blood 2002;100(7):2292-302 (PMID: 12239137).

20. Bennett JM, Catovsky D, Daniel MT, et al. Proposed revised criteria for the classification of acute myeloid leukemia A report of the French-American-British Cooperative Group. Ann Intern Med 1985;103(4):620-5 (PMID: 3862359).

21. Kudo Y, Boyd C, Sargent I, Redman C. Hypoxia alters expression and function of syncytin and its receptor during trophoblast cell fusion of human placental BeWo cells: implications for impaired trophoblast syncytialisation in pre-eclampsia. Biochim Biophys Acta 2003;1638(1):63-71 (PMID: 12757936).

22. Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25(4):402-8 (PMID: 11846609).

23. Stetler-Stevenson M, Arthur DC, Jabbour N, et al. Diagnostic utility of flow cytometric immunophenotyping in myelodysplastic syndrome. Blood 2001;98(4):979-87 (PMID: 11493442), comment in (PMID: 11783436).

24. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001;414(6859):105-11 (PMID: 11689955).

25. Adolfsson J, Månsson R, Buza-Vidas N, et al. Identification of Flt3+ Lympho-Myeloid Stem Cells Lacking Erythro-Megakaryocytic Potential: A Revised Road Map for Adult Blood Lineage Commitment. Cell 2005;121(2):295-306 (PMID: 15851035).

26. Liepert A, Grabrucker C, Kremser A, et al. Quality of T-cells after stimulation with leukemia-derived dendritic cells (DC) from patients with acute myeloid leukemia (AML) or myeloid dysplastic syndrome (MDS) is predictive for their leukemia cytotoxic potential. Cell Immunol 2010;265(1):23-30 (PMID: 20663492).

27. Driessens G, Gordower L, Nuttin L, et al. Therapeutic efficacy of antitumor dendritic cell vaccinations correlates with persistent Th1 responses, high intratumor CD8+ T cell recruitment and low relative regulatory T cell infiltration. Cancer Immunol Immunother 2008;57(12):1745-56 (PMID: 18369621).

28. Pui CH, Ip SH, Dodge RK, et al. Serum levels of CD8 antigen in childhood lymphoid malignancies: a possible indicator of increased suppressor cell activity in poor-risk patients. Blood 1988; 72(3):1015-21 (PMID: 2970871).

29. Denner J, Norley S, Kurth R. The immunosuppressive peptide of HIV-1: functional domains and immune response in AIDS patients. AIDS 1994;8(8):1063-72 (PMID: 7986401).

Supplementary Table

Table 1. Additional details on flow cytometry.

Flow cytometer manufacturer and model	**BD Biosciences FACSCantoTMII flow cytometer (BD Biosciences, San Jose, CA, USA)**
Flow cell and fluidics	**Fixed-alignment cuvette flow cell. The cells and fluidics had not been altered from the original manufacturer**
Light sources	**Two-lasers: 488-nm Coherent® SapphireTM solid state (20 mW) and 633-nm JDS UniphaseTM HeNe air-cooled (17 mW). The light sources had not been altered from the original manufacturer.**
Excitation optics configuration	**The configuration had not been altered from the original manufacturer**
Optical filters	**The filters were from the original manufacturer**
Optical detectors	**The detectors were from the original manufacturer**
Detectors/amps	**Parameter Detector Voltage Mode** **P1 FSC 286 Lin** **P2 SSC 434 Lin** **P3 FL1 473 Log** **P4 FL2 550 Log** **P5 FL3 530 Log** **P6 FL4 650 Log** **Threshold** **Primary parameter: FSC** **Value: 40** **Secondary parameter FL3** **Value: 170** **Compensation** **FL2 - 49% FL1** **FL3 - 3.5% FL1** **FL4 - 0.0% FL1** **FL1 - 0.4% FL2** **FL3 - 10.0% FL2** **FL4 - 0.0% FL2** **FL1 - 0.1% FL3** **FL2 - 1.3% FL3** **FL4 - 4.3% FL3** **FL3 - 0.1% FL4**
Amplifier settings	**The amplifier settings were calibrated monthly using BD Cytometer Setup Tracking Beads (no. 641319, BD Biosciences)**
Data transformation	**BD FACSDiva™ software (version 6.1.2) was used to transform the data as follows:** **- "*Select which parameters to display with logarithmic scaling*": All fluorescence parameters** **- "*Linear channel number corresponding to left edge of log display*": 3** **- "*Number of decades to display log-converted data*": 5 for pulse area parameters, 4 for pulse height parameters** **- "*Log transformation*": enabled; "*number of decades*": 4.5; "*additional negative display size*": 0; "*width basis*": -10**
Gating details	**An orthogonal side scatter (SS) versus CD45 plot was used to segregate four lymphocytic subsets: granulocytes, lymphoblasts, lymphocytes, and monocytes. Applying the isotype controls, voltage, and compensation, the flow cytometer was configured so the leukocyte subsets were appropriately positioned on the dot plots. The mean fluorescence intensities (MFIs) for syncytin-1 in each leukocytic subset (i.e, granulocytes, lymphoblasts, lymphocytes, and monocytes) were calculated from the leukocytic distribution pattern in the SS versus CD45 plot**