knowledge about the key players regulating the mobilization of leukemia cells is still severely limited [1,9,10]. Considering the progress in cell biological and genetic classification of leukemia cells, one approach to address the unsolved issues is the identification of an appropriate circulating molecule(s) released directly from the leukemic cells.

LR11 (also called SorLA or SORL1) is a type I membrane protein, from which a large soluble extracellular part, sLR11, is released by proteolytic shedding [11–14]. Substantial studies have shown that LR11 plays important roles both in intimal thickening and macrophage-foam cell formation in the process of atherosclerosis and in amyloidogenesis of Alzheimer disease [15,16]. The smooth muscle cell (SMC)-derived sLR11 causes macrophage infiltration in injured arteries by binding to urokinase-type plasminogen activator receptor (uPAR; CD87) on the cell surface [17,18]. Membrane-bound LR11 regulates the intracellular trafficking and processing of amyloid precursor protein (APP) by impairing the cleavage of APP through secretases in a way that leads to reduced levels of amyloid beta protein, the major component of amyloid plaques [19]. Thus, the levels of the membrane-bound form in neurons and of the soluble form in cerebrospinal fluid are highly associated with the pathogenesis of Alzheimer disease [20–22], and the circulating levels of the soluble form reflect the progression of atherosclerosis [23,24].

In addition, Zhang et al. reported high levels of LR11 mRNA in human $CD34^+CD38^-$ immature hematopoietic precursors [25]. However, little is known about the expression and the role(s) of LR11 and/or sLR11 in human hematopoietic cells. CD87 binds to and facilitates CD11b/CD18-mediated adhesion of human monocytes and induces proinflammatory signaling in human polymorphonuclear neutrophils [26]. Furthermore, CD87 is highly expressed in acute myeloid leukemia (AML) cells, especially myelo-monocytic subtypes, and patients with higher proportions of $CD87^+$ cells show a significantly lower remission rate and higher relapse risk [27,28]. Here, we investigated whether circulating sLR11 may represent the pathological conditions of undifferentiated leukemic cells.

2. Materials and methods

2.1. Antibodies and cells

Monoclonal antibodies (A2-2-3, M3) against LR11 were described previously [29]. M3 was used for flow cytometric analysis, and A2-2-3 for immunoblotting. Human leukemia cell lines, Daudi, MOLT-4, TALL-1, CCRF-SB, HL-60, ML-2, NB-4, RPMI8226, U937, and K562 were purchased from ATCC. The neuroblastoma cell line IMR32 was used as control cells expressing LR11 mRNA and protein, and releasing the soluble form as described [18,29]. All cultured cells were maintained in the media recommended by ATCC with 10% fetal bovine serum (FBS, Terumo). For mRNA and protein experiments, cells were used after incubation with serum-free media (1×10^6 cells/ml) for 30 h. For immunoblot analysis of sLR11 in the culture media, following serum starvation for 24 h, the cells were cultured with fresh serum-free media, and after 6 h the conditioned media were collected. The collected media were used for immunoblot analysis of sLR11 after concentration using Centricon-100 (Millipore). Before each experiments, cell viability was tested by trypan blue dye exclusion and confirmed the viability >95%.

2.2. Flow cytometry

Samples were analyzed on a JSAN desktop cell sorter (Bay Bioscience) with FlowJo software (Tree Star) as described [30]. Cells were stained with fluorescein isothiocyanate (FITC)-conjugated anti-LR11 monoclonal antibody M3 [29] in the presence or absence of antibodies against CD4, CD8, CD14, CD19, CD34, CD38, or CD11b (Becton Dickinson, San Jose, CA). For comparison of surface expression, mean fluorescence intensity (MFI) was evaluated. Surface expression of LR11 on peripheral leukocytes was analyzed in three healthy volunteers.

2.3. sLR11 ELISA

sLR11 levels were determined by the sandwich ELISA previously reported [29]. Briefly, samples (10 μl) diluted with sample buffer were reacted with the capture monoclonal antibody M3, and then incubated with the biotinylated rat monoclonal reporter antibody R14. The LR11-antibody complex was quantitated with horseradish peroxidase-conjugated streptavidin using purified LR11 protein as standard.

2.4. Immunoblotting

Cultured cells were washed three times with PBS, and collected in solubilization buffer (200 mmol/l Tris-maleate, pH 6.5, 2 mmol/l $CaCl_2$, 0.5 mmol/l PMSF, 2.5 μmol/l leupeptin and 1% Triton X-100) as described [24]. For immunoblotting, equal amounts of membrane protein or collected media were subjected to 10% SDS-PAGE after heating to 95 °C for 5 min as described under reducing conditions, and transferred to a nitrocellulose membrane. For immunodetection, A2-2-3 (1:500 dilution) was used, followed by peroxidase-conjugated anti-mouse IgG. Development was performed with the ECL detection reagents (Amersham Pharmacia, Piscataway, NJ). The signals were quantified by densitometric scanning using NIH image™ software.

2.5. Real-time quantitative PCR

Total RNA was prepared from cultured cells using an RNeasy kit (Qiagen, Valencia, CA) as described [24]. The methods for real-time quantitative PCR (qPCR) have been described [24]. For quantification of transcript levels, qPCR was performed using SYBR green PCR master mix and the PCR primers for LR11 mRNA (Hs00300475_s1, Applied Biosystems, Foster City, CA). mRNA amounts were normalized to levels of 18S ribosomal mRNA (Hs99999901_s1), which served as internal standard.

2.6. Patients

Flow cytometric analysis of LR11 and CD87 expression on leukemic cells was performed and MFI was evaluated in AML and acute lymphoblastic leukemia (ALL) patients between 2009 and 2011 in Chiba University Hospital. For sLR11 measurement, 139 subjects with various hematological diseases including 43 AML and 23 ALL patients diagnosed from 1999 to 2010 in Chiba University Hospital or affiliated hospitals and last followed-up in June 2010 were enrolled in this study, which was approved by the Human Investigation Review Committee of the Chiba University Graduate School of Medicine or affiliated hospitals. All acute leukemia patients were treated by chemotherapy according to the protocols established by the Japan Adult Leukemia Study Group (JALSG, http://www.jalsg.jp/english/01/e_index.html). Serum samples of patients with AML or ALL were collected at diagnosis and at remission, and subjected to analysis of sLR11 by ELISA. All clinical data were subjected to statistical analysis. One hundred serum samples from healthy volunteers were used for control.

2.7. Statistical analysis

Comparisons of serum sLR11 levels between patient and control samples, and between tertiles of peripheral blast proportions were evaluated by Dunnett's test. Serum sLR11 levels at diagnosis and at remission were compared using Wilcoxon's signed-rank test. As for variable selection, the stepwise procedure was set to

a threshold of 0.15 for inclusion or exclusion in linear regression analyses. All comparisons were planned and the tests were two-sided. A P value of <0.05 was considered to be statistically significant. All statistical analyses were performed using the JMP (ver 7.0.2, SAS Institute Inc., Cary, NC) and SAS (ver 9.2, SAS Institute Inc.) programs.

3. Results

3.1. Surface expression of LR11 on leukocytes and leukemic cells

First, we examined the expression of LR11 on leukocytes and leukemic cells by flow cytometry. Most $CD14^+$ peripheral monocytes express

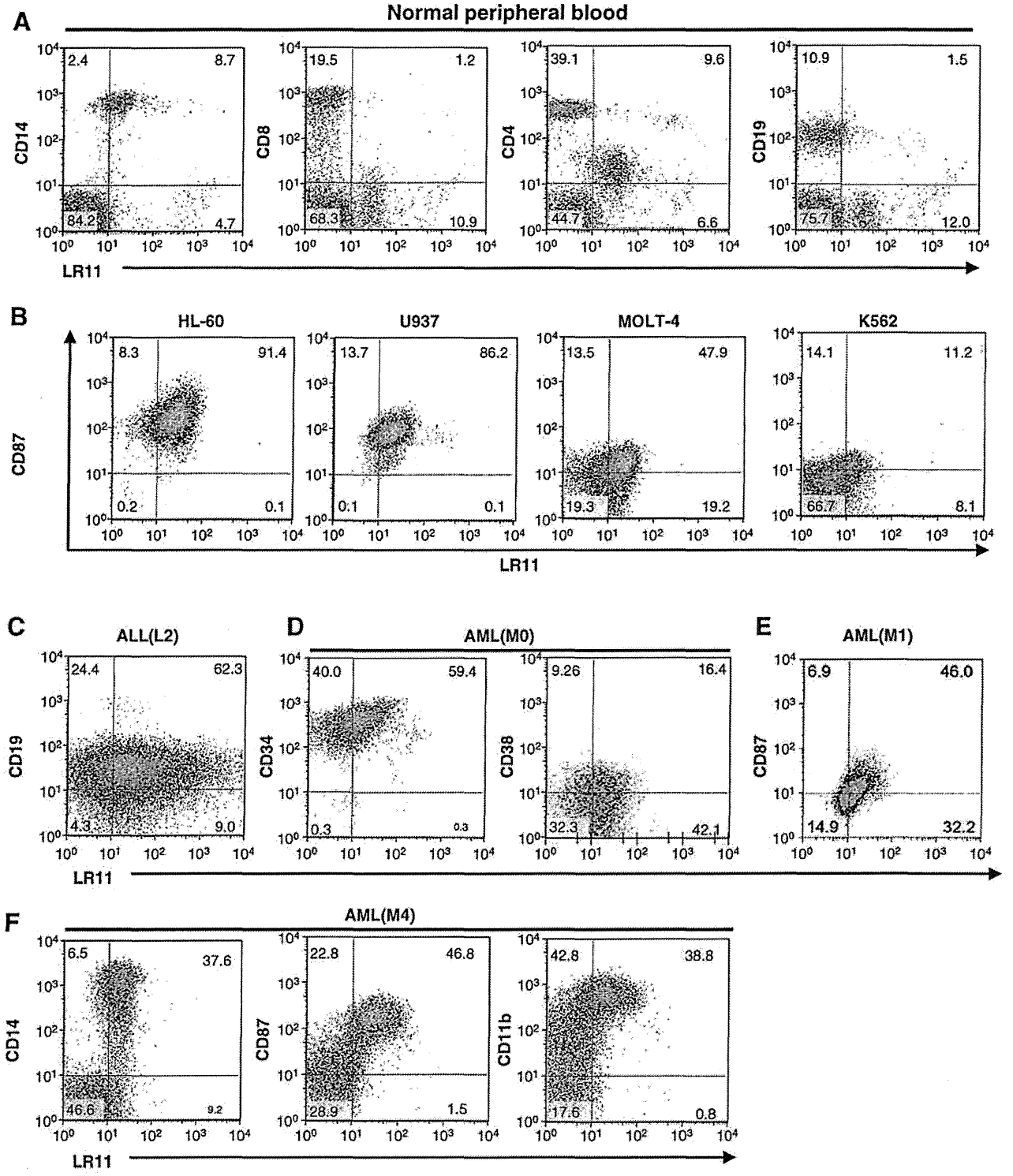

Fig. 1. Identification of LR11-expressing cells in peripheral blood leukocytes and leukemia cells by flow cytometry. (A) Peripheral blood mononuclear cells obtained from a normal subject were stained with the indicated antibodies. (B) Co-expression of LR11 and CD87 on leukemic cell lines. Representative data were shown. (C–F) Co-expression of LR11 and CD87 on leukemic cells. Bone marrow or peripheral blood mononuclear cells obtained from leukemia patients were stained with the indicated antibodies. The percentage of cells in each quadrant is indicated.

Table 1
Surface expression of LR11 and uPAR (CD87) on patients' leukemic cells.

Patient	FAB	LR11		uPAR (CD87)		Double positive	
	Classification	(%)	Positivity[a]	(%)	Positivity[a]	(%)	Positivity[a]
AML							
1#	M0	59.4	+	16.0	+	12.9	+
2	M1	30.9	+	24.2	+	14.3	+
3[b]	M1	78.2	+	52.9	+	46.0	+
4	M1	64.9	+	34.2	+	24.7	+
5	M2	33.7	+	8.2	−	2.5	−
6	M2	71.0	+	34.3	+	28.7	+
7	M2	18.7	+	8.0	−	4.1	−
8	M2	5.8	−	3.9	−	1.4	−
9	M2	73.7	+	42.1	+	33.0	+
10	M2	24.9	+	1.0	−	0.6	−
11	M2	22.7	+	38.9	+	17.6	+
12	M2	51.1	+	20.0	+	13.1	+
13	M2	8.7	−	2.8	−	1.9	−
14	M2	62.2	+	25.2	+	20.5	+
15	M3	66.5	+	37.0	+	30.6	+
16	M3	72.6	+	9.0	−	7.8	−
17	M3	14.4	+	1.0	−	0.4	−
18[b]	M4	48.6	+	69.6	+	46.8	+
19	M4	25.0	+	13.0	+	10.9	+
20	M4	89.3	+	61.8	+	56.1	+
21	M4	34.9	+	10.1	+	6.4	−
22	M4	25.7	+	33.1	+	13.1	+
23	M4	15.5	+	12.0	+	4.6	−
24	M6	92.1	+	31.5	+	29.1	+
Mean±SD		45.1±25.7	22/24 (91.7%)	24.5±19.1	17/24 (70.8%)	16.0±33.8	15/24 (62.5%)
ALL							
1[b]	L2	76.3	+	20.6	+	14.5	+
2	L2	9.5	−	6.6	−	1.1	−
3	L2	47.3	+	26.3	+	11.7	+
4	L2	63.0	+	1.6	−	0.8	−
5	L2	60.2	+	22.5	+	17.6	+
6	L2	12.7	+	5.2	−	1.0	−
7	L2	90.8	+	5.4	−	5.4	−
8	L2	93.3	+	59.0	+	56.7	+
Mean±SD		56.6±32.1	7/8 (87.5%)	18.4±18.9	4/8 (50%)	13.6±18.6	4/8 (50%)

[a] Data cut-off level is 10%.
[b] Patients with AML Nos. 1, 3, and 18 and ALL No. 1 are corresponding to Fig. 1D, E, F, and C, respectively.

LR11 (Fig. 1A), whereas the expression is much lower in most T cells ($CD4^+$, $CD8^+$), B cells ($CD19^+$), or granulocytes (data not shown). The leukemia cell lines HL-60 (promyelocytic), NB-4 (promyelocytic), U937 (monocytic), ML-2 (myeloblastic), MOLT-4 (lymphoblastic), and CCRF-SB (lymphoblastic) express LR11 on their cell surface; the expression on K562 (chronic myelogenous) and TALL-1 (lymphoblastic) was lower (Fig. 1B). In addition, most of LR11 positive cells express CD87 on their cell surface. Thus, LR11 is significantly co-expressed with CD87 on the surface of leukemic cells.

Next, we explored the expression of LR11 on the surface of leukemia cells in 24 AML patients and 8 ALL patients. Representative data were shown in Fig. 1C–F. The $CD19^+$ cells in ALL (FAB L2) showed the highest levels of LR11, within a wide range (Fig. 1C). Over 50% of $CD34^+$ cells in AML (FAB M0) were LR11-positive, whereas LR11-positive blasts predominated in the $CD38^-$ fraction (Fig. 1D). Some leukemic cells co-expressed both LR11 and CD87 (Fig. 1E). Among AML patients, in FAB M4 the majority of mononuclear cells with high CD14-expression were LR11-positive with a narrow LR11 expression range and 97% and 98% of the LR11-expressing mononuclear cells were also positive for CD87 and CD11b, respectively (Fig. 1F). In total, 22 out of 24 AML cases (91.7%) and 7 out of 8 ALL cases (87.5%) were positive for LR11 on their leukemic cells, whereas 70.8% of AML cases and 50% of ALL cases were positive for CD87 (Table 1). In these patients, a significant portion of CD87-positive cells also co-expressed LR11 on their cell surface.

3.2. Correlation of LR11 levels and released sLR11 in leukemic cell lines

Next, we examined the significance of sLR11 from immature leukemia cells. Quantitative PCR and ELISA revealed differences in LR11 expression in mRNA and released sLR11 of 10 leukemia cell lines of different origins, respectively (Table 2). There was a clear-cut correlation between the levels of mRNA and released sLR11 protein (Fig. 2A).

Table 2
The relationship of mRNA expression, surface LR11 and CD87, and levels of cell-released sLR11 in various kinds of leukemia-derived cell lines.

Cell lines	LR11 mRNA (qPCR)[a]	sLR11 in culture media (ELISA)[a]	Mean fluorescence intensity	
			LR11	CD87
Daudi	3.7±0.3	2.8±0.6	7.8	10.5
MOLT-4	2.2±0.1	2.9±0.2	16.6	13.3
TALL1	0.4±0.2	0.4±0.1	5.4	5.7
CCRF-SB	7.9±0.8	5.1±0.7	14.5	10.6
HL-60	3.1±0.5	3.6±0.4	28.2	205.0
ML-2	3.8±0.4	3.0±0.8	46.5	126.0
NB-4	2.7±0.2	1.8±0.1	30.3	32.8
RPMI8226	1.0±0.1	0.4±0.2	10.3	16.9
U937	4.1±0.7	3.2±0.6	26.5	118.0
K562	0.1±0.0	0.0±0.0	10.6	8.9

[a] LR11 mRNA levels and sLR11 levels in the culture media are expressed relative to that in IMR32. Data are presented as mean±SD (n=3).

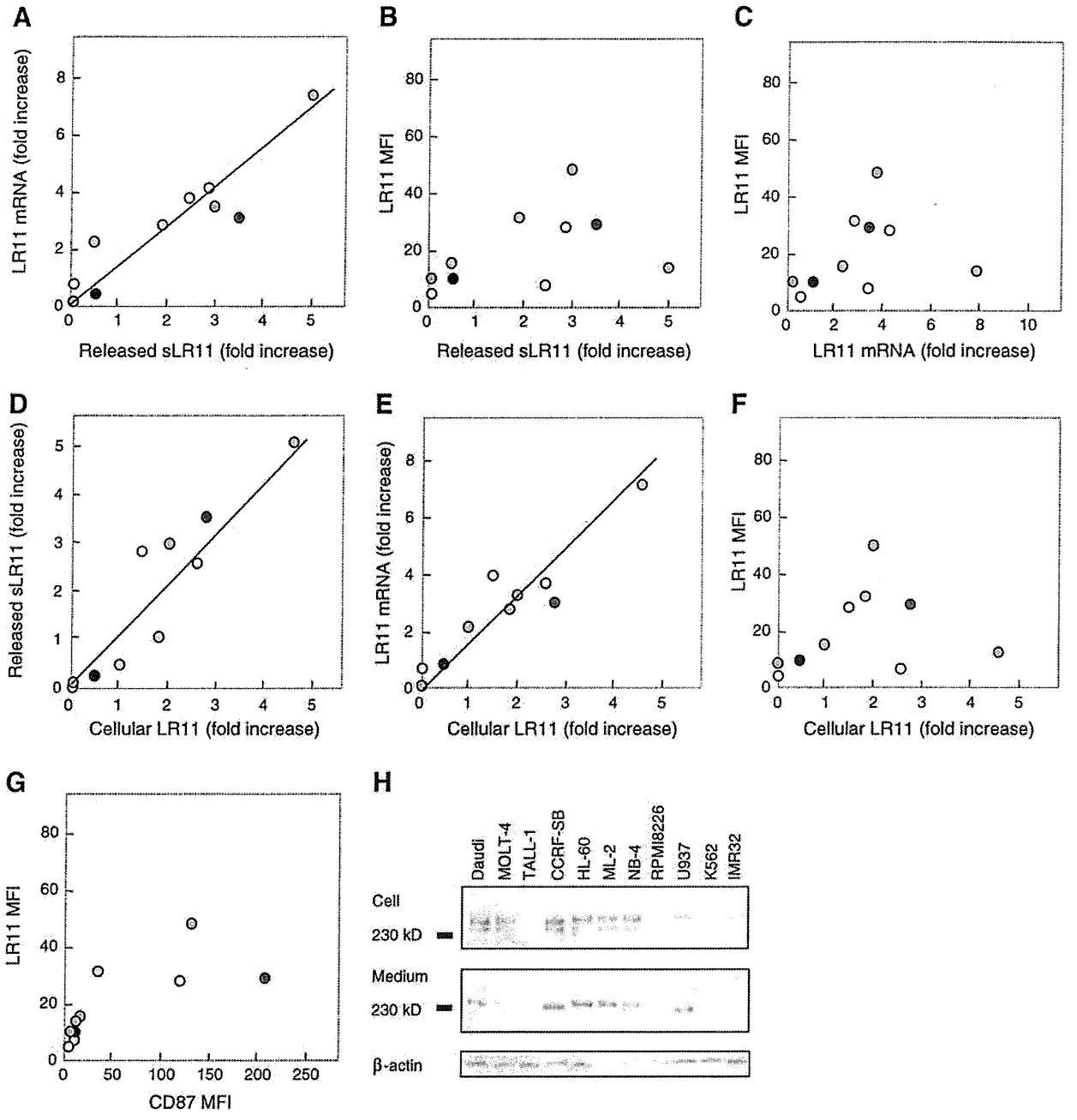

Fig. 2. The relationship between mRNA, cell protein, surface LR11, and levels of cell-released sLR11 in various kinds of leukemia-derived cell lines. (A–G) The relationships between the levels of LR11 mRNA and sLR11 protein (A), between the levels of surface LR11 (MFI) and sLR11 protein (B), between the levels of surface LR11(MFI) and LR11 mRNA (C), between the levels of sLR11 and cellular LR11 protein (D), between the levels of LR11 mRNA and cellular LR11 protein (E), between the levels of surface LR11 (MFI) and cellular LR11 protein (F), and between the levels of surface LR11 (MFI) and CD87 (MFI) (F) were analyzed using the results shown in Table 2 and (H). The signals for cellular LR11 in (H) were quantified by densitometric scanning using NIH software. The Spearman's rank correlation coefficients and P values are $r=0.94$ and $P<0.001$ in (A), $r=0.95$ and $P<0.001$ in (D), and $r=0.93$ and $P<0.001$ in (E), respectively. There were no significant correlations in (B), (C), (F) and (G). (H) LR11 protein in cells (1×10^6 cells/ml) after serum starvation for 30 h, and sLR11 in the serum-free media collected for 6 h. LR11 was detected as signals migrating at around 250 kDa by immunoblot analysis with antibody A2-2-3 directed against LR11. The deduced molecular weights of the membrane-bound form and the soluble form are approximately 250 and 230 kDa, respectively (17–19). Data are representative of 3 independent experiments.

On the other hand, surface expression levels of LR11 (expressed as MFI) were not significantly correlated with the levels of released sLR11 protein or LR11 mRNA (Fig. 2B and C, respectively). LR11 has been shown to localize in intracellular vesicles and to be released through shedding by proteinases from the membrane of neurons and smooth muscle cells [14–16,22,24]. We therefore analyzed the expression levels of cell-associated LR11 in the above cultured cells and the released protein by immunoblotting (Fig. 2H). There were significant correlations between cellular LR11 levels and the levels of released sLR11 or LR11 mRNA (Fig. 2D and E). On the other hand, there was no clear correlation between the cellular LR11 levels and surface LR11 expression levels (Fig. 2F), mainly because a few cell lines showed extremely high or low MFI of LR11 on the cell surface (e.g., CCRF-SB and Daudi), although the released sLR11 levels were dependent on the cellular LR11 levels in most of cell lines (Fig. 2H). The surface LR11 levels were not strictly restricted by the surface CD87 expression levels (Table 2, Fig. 2G). Thus, the release of LR11 by leukemia cell lines of different origins appeared to be the result of a dual regulation by transcriptional activity and proteinase shedding.

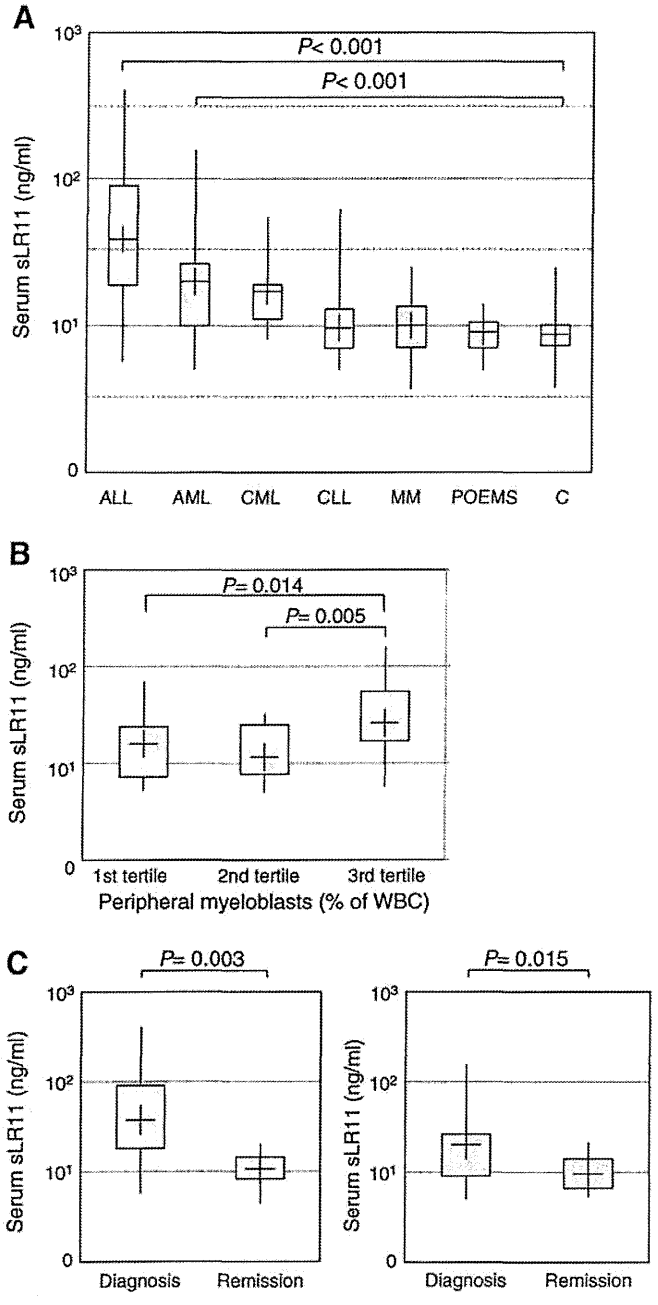

Fig. 3. Circulating sLR11 levels in patients with acute leukemias. (A) Upper and lower edges of boxes indicate upper and lower quartiles, respectively; upper and lower bars indicate maximum and minimum, respectively; short horizontal lines represent median values. MM indicates multiple myeloma; POEMS, POEMS syndrome; C, control subjects without diagnosed hematological diseases. (B) Serum sLR11 levels at diagnosis in patients with AML, grouped by tertiles (n = 14 each) of peripheral myeloblasts (% of WBC). Peripheral myeloblast ranges for 1st, 2nd, and 3rd tertiles were <20.0%, 23.0% to 64.0%, and >67.5%, respectively. P values were determined by Dunnett's test. (C) The paired data at diagnosis and at remission are shown for all patients with ALL (left) and AML (right).

3.3. Serum sLR11 in acute leukemia

Next, serum sLR11 levels were determined in 139 patients with diagnosed acute leukemias and other hematological diseases (Supporting Table S1, Fig. 3). sLR11 levels of acute leukemia patients at diagnosis were significantly increased over those in subjects without hematological disease (n = 100; median, 7.7 ng/ml; range, 4.7–17.2, S.D., 3.3). In ALL, we determined a median of 37.1 ng/ml (range, 5.7–407.0, S.D., 93.5; P<0.001) and in AML, a median of 20.0 ng/ml (range, 5.0–157.5, S.D., 29.1; P<0.001) (Fig. 3A). sLR11 levels in patients with chronic myeloid leukemia (CML; median, 17.1 ng/ml; range, 8.1–55.0, S.D., 11.1), chronic lymphocytic leukemia (CLL; median, 9.6 ng/ml; range, 5.0–62.0, S.D., 11.6), multiple myeloma (MM, median, 10.0 ng/ml; range, 3.7–25.0, S.D., 4.8), and POEMS syndrome (median, 9.0 ng/ml; range, 4.9–14.0, S.D., 2.7) were not significantly different from controls. To identify factors influencing serum sLR11 levels at diagnosis, we used Stepwise regression models including age, peripheral WBC count, peripheral blast population, peripheral blast count, bone marrow blast population, and serum LDH level. The correlation between peripheral blast proportion in AML and ALL and the sLR11 level was independent of other prognostic risk factors at diagnosis (Table 3). Among 42 AML patients, sLR11 levels of subjects in the highest tertile of peripheral blast proportion (>67.5% of WBC) were 2.44- and 3.05-fold higher than those in the middle (23.0–64.0% of WBC) and lowest tertiles (<20.0% of WBC), respectively (Fig. 3B). Paired sample analysis of patients with AML and ALL who achieved complete remission (CR) showed significantly decreased sLR11 levels compared to those at diagnosis, and most notably, in CR the levels were all below 20 ng/ml (Fig. 3C). These data suggest that sLR11 levels indeed are representative of the pathological conditions of patients with acute leukemias.

Table 3
Stepwise regression analysis for factors influencing serum sLR11 levels at diagnosis.

Variable	Estimate	Standard error	Adjusted β-coefficients	R^2	P value
AML					
Peripheral blast proportion (% of WBC)	0.38	0.12	0.45	0.21	0.003
ALL					
Peripheral blast proportion (% of WBC)	1.97	0.61	0.72	0.34	0.004

Stepwise regression models included age, peripheral WBC count, peripheral blast population, peripheral blast count, bone marrow blast population, and serum LDH level. The stepwise procedure was set to a threshold of 0.15 for inclusion and exclusion.

4. Discussion

This study has revealed that LR11 is a novel surface molecule for monocytes. Furthermore, LR11 as well as CD87, a partner of sLR11, were expressed on the surface of leukemic cells in AML and ALL for formation of a complex mediating functional intracellular signal activation. Since the patient data indicate a tight association of sLR11 levels with peripheral blast proportions independently of other prognostic risk factors, the level of sLR11 at diagnosis may serve as novel candidate information reflecting the status and efficacy of treatment of acute leukemias.

Zhang et al. reported that the high level of LR11 mRNA expression was found in peripheral white blood cells, and among white blood cells, LR11 was highly expressed in neutrophils and lower in lymphocytes, but not in monocytes. In contrast to these data, we found by flow cytometry that the surface expression of LR11 was higher in monocytes than in neutrophils and lymphocytes. LR11 is mostly localized in endosome vesicles in many kinds of cells [15,16], in which LR11 is believed to be important for the transport of intracellular proteins, such as APP in neuron [16]. The granular staining pattern in the cells has suggested that the soluble form of LR11 exists in addition to membrane-bound form in the vesicles. Thus, we have shown that the soluble form of LR11 is active for the complex formation with CD87 on the membrane as membrane bound form [12,17,18]. These data suggested that the levels of the released form of LR11 and of the surface form are possibly regulated by the combined activities of mRNA transcription and post-translational protein transport with subsequent proteinase-mediated shedding from the surface. In this context, LR11 has been shown to be released from neurons and

SMCs by the potential actions of proteinases including ADAM17/TACE [11–14].

Questions remain about the mechanisms underlying enhanced levels of surface LR11 and circulating sLR11 in leukemia cells of many different, but not all, origins. We have previously shown that sLR11 accelerates the co-localization of uPAR (CD87) and integrin $\alpha_v\beta_3$ in the membrane of SMCs [24]. Moreover, uPAR can regulate the adhesion of HSPCs to the bone marrow microenvironment and the homing and engraftment of HSPCs, in part via integrin $\alpha_4\beta_1$ [31]. In this study, most of leukemic cells and leukemic cell lines co-express LR11 and uPAR on their cell surface, although there were also leukemic cells without the harmonized expressions of both molecules (see Tables 2 and 3, and Fig. 2). Thus, sLR11 might interact with uPAR in association with cell-specific integrin isoforms to regulate mobility-related functions in leukemia cells of some origins.

Since sLR11 levels are associated with the peripheral blast proportion in acute leukemias, but are not elevated in chronically proliferative diseases such as CML, CLL, and MM, circulating sLR11 levels are unlikely to be simply associated with proliferating cell numbers. Rather, sLR11 levels appear to be predictive for pathogenic properties of immature blasts, including their migration and attachment activities. Recombinant sLR11 induces adhesion and migration of HL-60 and U937 cells, and these actions are involved in G-CSF-mediated leukocyte mobilization (Shimizu et al., unpublished observations). The fact that sLR11 is not increased in POEMS syndrome, in which extremely high levels of circulating VEGF cause vessel injury [32], suggests that increased sLR11 levels in acute leukemias are not a result of vascular injury, but rather a consequence of the enhanced release from leukemic cells.

Currently, the limitation of this study is the small number of patients available for studies, particularly for that of ALL. Further extensive flow-cytometric analysis of leukemic cells in AML patients with each FAB subtype as well as in ALL patients, in relation to sLR11 levels in the circulation, is expected to reveal the pathophysiological significance of sLR11 as a leukemia marker.

In summary, we have identified sLR11 as a novel circulating molecule directly released from leukemic cells in acute leukemias. Considering the lack of circulating molecules shed from the surface of leukemia cells, a prospective clinical trial testing the efficacy of sLR11 as a candidate biomarker for clinical characteristics and chemotherapeutic outcome in acute leukemia patients is now in progress.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cca.2012.06.025.

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References

[1] Dohner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood 2010;115:453–74.
[2] Litzow MR. Evolving paradigms in the therapy of Philadelphia-chromosome-negative acute lymphoblastic leukemia in adults. Hematology Am Soc Hematol Educ Program 2009:362–70.
[3] Pui CH, Robison LL, Look AT. Acute lymphoblastic leukaemia. Lancet 2008;371:1030–43.
[4] Massberg S, Schaerli P, Knezevic-Maramica I, et al. Immunosurveillance by hematopoietic progenitor cells trafficking through blood, lymph, and peripheral tissues. Cell 2007;131:994–1008.
[5] Lane SW, Scadden DT, Gilliland DG. The leukemic stem cell niche: current concepts and therapeutic opportunities. Blood 2009;114:1150–7.
[6] Kessinger A, Sharp JG. The whys and hows of hematopoietic progenitor and stem cell mobilization. Bone Marrow Transplant 2003;31:319–29.
[7] Selleri C, Montuori N, Ricci P, et al. Involvement of the urokinase-type plasminogen activator receptor in hematopoietic stem cell mobilization. Blood 2005;105:2198–205.
[8] Matsunaga T, Takemoto N, Sato T, et al. Interaction between leukemic-cell VLA-4 and stromal fibronectin is a decisive factor for minimal residual disease of acute myelogenous leukemia. Nat Med 2003;9:1158–65.
[9] Binet JL, Caligaris-Cappio F, Catovsky D, et al. Perspectives on the use of new diagnostic tools in the treatment of chronic lymphocytic leukemia. Blood 2006;107:859–61.
[10] Radich JP, Zelenetz AD, Chan WC, et al. NCCN task force report: molecular markers in leukemias and lymphomas. J Natl Compr Canc Netw 2009;7(Suppl. 4):S1–S34 [quiz S5-6].
[11] Yamazaki H, Bujo H, Kusunoki J, et al. Elements of neural adhesion molecules and a yeast vacuolar protein sorting receptor are present in a novel mammalian low density lipoprotein receptor family member. J Biol Chem 1996;271:24761–8.
[12] Ohwaki K, Bujo H, Jiang M, Yamazaki H, Schneider WJ, Saito Y. A secreted soluble form of LR11, specifically expressed in intimal smooth muscle cells, accelerates formation of lipid-laden macrophages. Arterioscler Thromb Vasc Biol 2007;27:1050–6.
[13] Jacobsen L, Madsen P, Moestrup SK, et al. Molecular characterization of a novel human hybrid-type receptor that binds the alpha2-macroglobulin receptor-associated protein. J Biol Chem 1996;271:31379–83.
[14] Hermey G, Sjogaard SS, Petersen CM, Nykjaer A, Gliemann J. Tumour necrosis factor alpha-converting enzyme mediates ectodomain shedding of Vps10p-domain receptor family members. Biochem J 2006;395:285–93.
[15] Bujo H, Saito Y. Modulation of smooth muscle cell migration by members of the low-density lipoprotein receptor family. Arterioscler Thromb Vasc Biol 2006;26:1246–52.
[16] Willnow TE, Petersen CM, Nykjaer A. VPS10P-domain receptors — regulators of neuronal viability and function. Nat Rev Neurosci 2008;9:899–909.
[17] Zhu Y, Bujo H, Yamazaki H, et al. LR11, an LDL receptor gene family member, is a novel regulator of smooth muscle cell migration. Circ Res 2004;94:752–8.
[18] Zhu Y, Bujo H, Yamazaki H, et al. Enhanced expression of the LDL receptor family member LR11 increases migration of smooth muscle cells in vitro. Circulation 2002;105:1830–6.
[19] Rogaeva E, Meng Y, Lee JH, et al. The neuronal sortilin-related receptor SORL1 is genetically associated with Alzheimer disease. Nat Genet 2007;39:168–77.
[20] Ikeuchi T, Hirayama S, Miida T, et al. Increased levels of soluble LR11 in cerebrospinal fluid of patients with Alzheimer disease. Dement Geriatr Cogn Disord 2010;30:28–32.
[21] Schmidt V, Baum K, Lao A, et al. Quantitative modelling of amyloidogenic processing and its influence by SORLA in Alzheimer's disease. EMBO J 2012;31:187–200.
[22] Alexopoulos P, Guo LH, Tsolakidou A, et al. Interrelations between CSF soluble AbetaPPbeta, amyloid-beta 1–42, SORL1, and Tau levels in Alzheimer's disease. J Alzheimers Dis 2012;28:543–52.
[23] Takahashi M, Bujo H, Jiang M, Noike H, Saito Y, Shirai K. Enhanced circulating soluble LR11 in patients with coronary organic stenosis. Atherosclerosis 2010;210:581–4.
[24] Jiang M, Bujo H, Ohwaki K, et al. Ang II-stimulated migration of vascular smooth muscle cells is dependent on LR11 in mice. J Clin Invest 2008;118:2733–46.
[25] Zhang X, Dormady SP, Basch RS. Identification of four human cDNAs that are differentially expressed by early hematopoietic progenitors. Exp Hematol 2000;28:1286–96.
[26] Sitrin RG, Pan PM, Harper HA, Todd III RF, Harsh DM, Blackwood RA. Clustering of urokinase receptors (uPAR; CD87) induces proinflammatory signaling in human polymorphonuclear neutrophils. J Immunol 2000;165:3341–9.
[27] Atfy M, Eissa M, Salah HE, El Shabrawy DA. Role of urokinase plasminogen activator receptor (CD87) as a prognostic marker in acute myeloid leukemia. Med Oncol 2011 [Epub June 2011].
[28] Graf M, Reif S, Hecht K, Pelka-Fleischer R, Pfister K, Schmetzer H. High expression of urokinase plasminogen activator receptor (UPA-R) in acute myeloid leukemia (AML) is associated with worse prognosis. Am J Hematol 2005;79:26–35.
[29] Matsuo M, Ebinuma H, Fukamachi I, Jiang M, Bujo H, Saito Y. Development of an immunoassay for the quantification of soluble LR11, a circulating marker of atherosclerosis. Clin Chem 2009;55:1801–8.
[30] Oguro H, Yuan J, Ichikawa H, et al. Poised lineage specification in multipotential hematopoietic stem and progenitor cells by the polycomb protein Bmi1. Cell Stem Cell 2010;6:279–86.
[31] Tjwa M, Sidenius N, Moura R, et al. Membrane-anchored uPAR regulates the proliferation, marrow pool size, engraftment, and mobilization of mouse hematopoietic stem/progenitor cells. J Clin Invest 2009;119:1008–18.
[32] Watanabe O, Arimura K, Kitajima I, Osame M, Maruyama I. Greatly raised vascular endothelial growth factor (VEGF) in POEMS syndrome. Lancet 1996;347:702.