の IVIG 治療に反応しなかったもの：不応群 7 例であった。さらに年齢をマッチさせた 5 例を対照群とした。IVIG 前（3〜7 病日）、IVIG 後 14 病日前後（10〜17 病日）、回復期（25〜36 病日）に静脈採血にて検体を採取した。急性期のバイオマーカーとして IL-1β、IL-2、IL-4、IL-5、IL-6、IL-8、IL-10、IL-17、IFN-γ、MCP-1、PDGF-BB、RANTES、TNF-α、VEGF を測定し sLR11 との関連を検討した。

全例初期治療として IVIG 療法とアスピリン内服を行った。初回 IVIG 不応例に対する追加治療として全例 2 回目の IVIG 投与を行った。不応群に対して 3 回目の IVIG 投与、ステロイド内服、ステロイドパルス療法、ウリナスタチン療法を行った。

2）川崎病遠隔期
発症 1 年以上の川崎病遠隔期患者 88 例を対象とした。急性期から冠動脈の異常を認めないもの（一過性拡大を含む）：no CAL 群 18 例、CAL が消失し造影上正常化したもの：退縮群 12 例 、後遺症として瘤、狭窄、閉塞等の病変が残存するもの：CAL 群 58 例であった。さらに対照群 15 例とした。外来受診時の静脈採血、あるいは心臓カテーテル検査時にシースまたはカテーテルから検体を採取した。遠隔期の血管障害バイオマーカーとして MCP-1、 TNF-α、MMP-9、PAI-1、IL-6、PDGF-BB、ICAM-1、E-Selectin、高感度 CRP を測定し sLR11 との関連を検討した。

（倫理的側面への配慮）
本研究の目的、内容につき、別紙資料を用いて両親などの代諾者に平易に説明し、別紙文章で同意を得た。また、年齢に応じ可能な限り患者本人にも理解し了承を得た。対象者が 16 歳以上の場合には、代諾者とともに、被験者からの同意も得ることとした。

C. 研究結果
1）川崎病急性期
IVIG 治療前 sLR11 値は、不応群で対照群、奏効群より有意に高値であった（不応群、 19.5 ± 5.8 ng/nl; 奏効群、11.8 ± 3.1ng/ml, p<0.001）（図 1）。IVIG 治療前 sLR11 のカットオフ値を 18.0ng/ml とすると、IVIG 不応予測の感度 71%、 特異度 100%であった。急性期患者全体の IVIG 治療前 sLR11 値（13.6±4.5ng/ml）は対照群（12.2±2.0ng/ml）と差がなかったが、14 病日前後（21.0±5.1ng/ml、p<0.0001）に有意に上昇し、回復期（17.6±4.6ng/ml、p=0.04）にも高値が持続した。また不応群はいずれの時期においても奏効群より有意に高値であった（ repeated ANOVA, p<0.0001）（図 2）。sLR11 以外のバイオマーカーのうち不応群の IVIG 前値が奏効群より有意に高値となるもの、いずれの時期も有意に高値をとるものはなかった。

2）川崎病遠隔期
CAL 群の sLR11 は、対照群、no CAL 群、退縮群と比べ有意に高値であった（対照群, 7.8±2.1ng/ml; no CAL 群、8.2±1.7ng/ml; 退縮群、8.5±2.1ng/ml; CAL 群、10.0± 2.3ng.ml）（図 3）。CAL 群のうち 9 人で上行大動脈（10.3±2.0ng/ml）、右房（10.4±2.0ng/ml）、冠静脈洞（10.9±2.0ng/ml）の 3 カ所の sLR11 は差がなかった。sLR11 は E-selectin（r=0.56,

p<0.0005)、高感度 CRP（r=0.52, p<0.001)
と正相関を認めた。

D. 考察

　川崎病急性期において IVIG 不応例の IVIG 前 sLR11 値は奏効例と比べ有意に高値を示し、IVIG 不応や CAL 発症の予測マーカーとなる可能性がある。sLR11 は IVIG 治療後に上昇し、回復期にも高値が持続する。また IVIG 不応例の sLR11 値は、奏効例と比べ治療前から回復期まで有意に高値をとる。これは従来の急性期バイオマーカーとは異なる動態であり、これらのマーカーとは違う観点から血管炎を反映している可能性がある。

　川崎病遠隔期において sLR11 は他の血管障害バイオマーカーに比し、良好に冠動脈病変の重症度を反映していた。採血部位による sLR11 に差がなかったことから全身の血管障害を反映している可能性がある。また E-selectin、高感度 CRP との有意な正相関があり、血管内皮障害や慢性炎症との関連が示唆された。

E. 結論

　sLR11 は川崎病急性期の重症度を反映し、γ グロブリン不応や冠動脈瘤形成の予測マーカーとなる可能性がある。また川崎病遠隔期の血管障害を反映するバイオマーカーとなる可能性がある。

F. 健康危険情報

なし

G. 研究発表

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H. 知的財産権の出願・登録状況（予定を含む。）

１．特許取得

　特になし

２．実用新案登録

　特になし

３．その他

　特になし

Ⅲ. 研究成果の刊行に関する一覧表

別紙4

研究成果の刊行に関する一覧表レイアウト

書籍

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Ⅳ．研究成果の刊行物・別冊

Alzheimer's
&
Dementia

ELSEVIER

β-Site amyloid precursor protein–cleaving enzyme 1 activity is related to cerebrospinal fluid concentrations of sortilin-related receptor with A-type repeats, soluble amyloid precursor protein, and tau

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Abstract

Background: β-Site amyloid precursor protein (APP)–cleaving enzyme 1 (BACE1) activity determines the rate of APP cleavage and is therefore the main driver of amyloid β production, which is a pathological hallmark of Alzheimer's disease (AD).

Methods: The present study explored the correlation between BACE1 activity and cerebrospinal fluid (CSF) markers of APP metabolism and axonal degeneration in 63 patients with mild AD and 12 healthy control subjects.

Results: In the AD group, positive correlations between BACE1 activity and soluble APP β, the APP sorting receptor sortilin-related receptor with A-type repeats (also known as SorLA or LR11), and tau were detected. BACE1 activity was not associated with amyloid β_{1-42} or soluble APP α concentrations in the AD group, and no associations between BACE1 activity and any of the protein concentrations were found in the control group.

Conclusion: Our results confirm the relevance of BACE1 and sortilin-related receptor with A-type repeats within the amyloid cascade and also provide a further piece of evidence for the link between amyloid and tau pathology in AD.
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Keywords: Alzheimer's disease; Dementia; Biomarker; Amyloid cascade; β-secretase

1. Background

The cerebral pathologic hallmarks of Alzheimer's disease (AD) include the extracellular accumulation of amyloid β (Aβ) plaques, synaptic and neuronal degeneration, and the presence of tau protein tangles [1]. Aβ plaques mainly consist of the 4-kDa Aβ peptide, which is generated by the enzymatic cleavage of the transmembrane amyloid precursor protein (APP). The first, and rate-limiting, APP cleavage step by the β-site APP-cleaving enzyme 1 (BACE1) [2] results in the production of the N-terminal soluble APP

(sAPP) β and a C-99 fragment, which is subsequently cleaved by the γ-secretase complex, resulting in Aβ. The alternative processing of APP by the α-secretases precludes the generation of Aβ because the cleavage site lies within the Aβ sequence; sAPPα is a product of this processing pathway [3]. The relevance of BACE1 in AD is supported by its increased expression and activity in the brain tissues [4,5] and cerebrospinal fluid (CSF) of patients with AD [6,7].

In addition to the secretases, the sortilin-related receptor with A-type repeats (SORL1, also termed LR11 or sorLA), a member of the apolipoprotein E and low-density lipoprotein receptor family [8,9], has emerged as another relevant regulator of APP processing; SORL1 is probably involved in the intracellular sorting of APP and its interactions with the secretases, including BACE1 [10]. According to recent

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evidence, SORL1 promotes the retention of APP in sub-cellular compartments that are less favorable for secretase processing, thereby reducing the extent of its proteolytic breakdown into both amyloidogenic and nonamyloidogenic products [11]. In line with this finding, the neuronal expression of SORL1 is dramatically decreased in the brains of patients with sporadic AD [12–14]. The large extracellular part of the receptor is released after endoproteolytic cleavage [15] and can therefore be measured in CSF; however, no general consensus has yet been reached regarding the effects of AD on SORL1 concentrations in CSF [16,17].

The aforementioned evidence and theoretical considerations suggest that BACE1 activity should be positively correlated with $A\beta_{1-42}$ and sAPPβ (but not sAPPα), and possibly also with tau as well as SORL1, concentrations in CSF. Some of these assumptions, such as the positive association between BACE1 activity and sAPPβ and tau concentrations, are backed by previous research, whereas others are not [18,19], which warrants replication. Furthermore, the correlation between the concentrations of SORL1, the encoding gene of which is among the strongest known genetic risk factors for sporadic AD [20], and BACE1 activity in CSF has not been studied thus far. The main aim of the present study was to provide evidence in relation to these issues.

2. Methods

2.1. Participant selection

Sixty-three patients with probable AD and available lumbar CSF samples were identified in the electronic database of the Department of Psychiatry and Psychotherapy at the Technische Universität München (Munich, Germany). Informed written consent was available for all patients; the study protocol was approved by the ethics committee of the faculty of medicine at the Technische Universität München. The clinical diagnoses had been established by consensus of two experienced clinicians according to National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association criteria for probable AD in conjunction with International Classification of Diseases (10th revision) criteria for mild AD dementia. The diagnostic workup included patient and proxy interviews, physical examination, psychometric testing, routine blood sampling, and structural imaging of the brain (magnetic resonance imaging or computed tomography). None of the patients showed signs of relevant cerebrovascular disease or any plausible cause for cognitive impairment other than AD. The psychometric assessment was based on the Consortium to Establish a Registry for Alzheimer's disease neuropsychological assessment battery, which incorporates the Mini-Mental State Examination. An additional group of 12 healthy control subjects, recruited at the Department of Neurology of the University of Bari in Italy, was included to explore the associations between the CSF protein levels in the absence of any relevant neurodegenerative

pathology. The control subjects had no subjective memory complaints and no history of cognitive impairment. They were independent in their activities of daily living and did not show any signs of a relevant psychiatric or neurological illness.

2.2. CSF sampling and analyses

CSF was collected in sterile polypropylene tubes using atraumatic cannulas placed in the L3/L4 or L4/L5 intervertebral space. The CSF was centrifuged (1800 $\times g$ at 4°C for 10 minutes) immediately after collection to remove cells. Aliquots of the remaining CSF supernatants were stored in polypropylene tubes at −80°C for further processing.

2.3. Determination of $A\beta_{1-42}$, tau, sAPPα, and sAPPβ levels

$A\beta_{1-42}$, total tau (Innogenetics, Ghent, Belgium), and sAPPα/sAPPβ (IBL, Gunma, Japan) levels in CSF were measured in duplicate using commercially available enzyme-linked immunosorbent assays (ELISAs) according to the manufacturers' instructions as described previously in greater detail [21-23].

2.4. BACE1 activity assay

BACE1 activity was measured using a time-resolved fluorescence activity assay based on SignalClimb technology (TruePoint Perkin Elmer, Turku, Finland) according to optimized manufacturer's instructions [7]. The synthetic TruePoint BACE1 substrate is a 10-amino acid–long peptide with a fluorescent europium chelate coupled to one end and a quencher of europium fluorescence (QSY7) coupled through lysine to the other end. The hydrolysis of the substrate's protein sequence CEVNLDAEFK by BACE1 results in a fluorescence signal proportional to the activity of BACE1. The fluorescence signal was measured at 37°C in a microplate reader using time-resolved fluorescence (FLUOstar Omega, BMG Labtech, Offenburg, Germany; excitation wavelength: 320 nm, emission wavelength: 615 nm) in black 96-well plates (Perkin Elmer, Turku, Finland) at a final volume of 27 µL, including 10 µL of CSF, 2 µL of dimethyl sulfoxide, and 15 µL of BACE1 substrate (0.80 nM/mL). The continuous measurement of BACE1 activity was started immediately after adding the CSF sample; BACE1 activity was defined as the maximal activity within the first 30 minutes. Each sample was measured at least four times to verify reproducibility. Proteinase inhibitors were added to block all non-BACE1 aspartyl protease activity.

2.5. SORL1 concentrations

SORL1 concentrations in CSF were quantified using ELISA in the laboratories of Sekisui Medical Co Ltd. (Ryugasaki, Japan) as described previously [24]. Briefly, 10 µL of CSF was diluted with 100 µL of sample buffer and added to

the plate coated with mouse monoclonal antibody M3 [25]. After incubating with the biotinylated rat monoclonal antibody R14, the SORL–antibody complex was reacted with horseradish peroxidase–conjugated streptavidin. A standard curve was constructed using purified SORL1 protein. The final absorbance of each sample was determined at 450 nm [24]. SORL1 concentrations were only determined in a subsample of 40 patients with probable AD and in the entire control group. The first published study using this assay [24] showed that purified SORL1 in CSF was immunologically identical to SORL1 from cell culture, strongly arguing that the ELISA measures the soluble form of the membrane-bound receptor.

2.6. Statistical analysis

Data were analyzed using the Predictive Analytics Software package version 18 (SPSS Inc., Chicago, IL) using two-sided tests. Normal distribution was checked using the Kolmogorov–Smirnov test. Correlations between BACE1 activity and CSF protein concentrations were investigated using the Pearson correlation coefficient. P values were regarded significant at a level of 5%; the false discovery rate (http://sdmproject.com/utilities/?show=FDR), which controls the expected proportion of incorrectly rejected null hypotheses (type I errors), was used to account for the error in multiple comparisons (i.e., results at $P_{corr} < .05$ were regarded significant).

3. Results

The characteristics of the study sample are shown in Table 1. The CSF concentrations of $A\beta_{1-42}$ and tau were in the expected range for this kind of sample [26]. The Mini-Mental State Examination score range confirmed the mild degree of dementia in the AD group and the lack of any objective

Table 1
Characteristics of the study groups

Variable	Control group	AD group
N	12	63
Age, years*	47.50 (13.70)	66.87 (9.39)
Age at onset, years*	na	62.83 (9.09)
Men/women	6:6	34:29
MMSE score*	30 (0.00)	22.54 (3.27)
BACE1 activity, FU/μL*	7468.43 (1966.75)	8757.01 (2636.36)
$A\beta_{1-42}$, ng/L*	708.33 (378.17)	540.48 (232.99)
Tau, ng/L*	125.58 (57.29)	605.21 (361.99)
sAPPα, ng/mL*	265.19 (218.35)	263.83 (145.58)
sAPPβ, ng/mL*	746.35 (519.92)	864.95 (405.522)
SORL1 ng/mL*	10.36 (2.61)	11.83 (4.74)[†]

Abbreviations: na, not applicable; nd, not done; AD, Alzheimer's disease; MMSE, mini-mental state examination; FU, fluorescence units; Aβ, amyloid β; sAPP, soluble amyloid precursor protein; BACE1, β-site amyloid precursor protein–cleaving enzyme 1; SORL1, sortilin-related receptor with A-type repeats.
*Mean (SD).
[†]N = 40.

cognitive impairment in the control group. In the AD group, there was a significant positive correlation after false discovery rate correction for multiple comparisons between BACE1 activity on the one hand and tau ($r = 0.30, P_{corr} = .04, N = 63$) as well as SORL1 concentrations ($r = 0.37, P_{corr} = .04, N = 40$) on the other hand (Figure 1). BACE1 activity was not correlated with sAPPα ($P_{corr} = .17$) or $A\beta_{1-42}$ ($P_{corr} = .91$) levels in the AD group. At a more liberal threshold of $P < .05$ uncorrected for multiple comparisons, BACE1 activity was also positively correlated with sAPPβ levels in patients with AD ($r = 0.26, P_{uncorr} = .05, N = 63$). BACE1 activity was not correlated with the levels of any of the CSF proteins in the control group (tau: $P_{corr} = .96$; $A\beta_{1-42}$: $P_{corr} = .75$; sAPPα: $P_{corr} = .55$; sAPPβ: $P_{corr} = .55$; SORL1: $P_{corr} = .90$).

4. Discussion

The present study explored the association between BACE1 activity, which is regarded as the rate-limiting factor of Aβ production, and the concentrations of several AD markers in CSF. Our findings partly confirm the results of previous research, but they also add new evidence to the existing literature, for example, by demonstrating a positive correlation between BACE1 activity and SORL1 concentration in CSF.

4.1. BACE1 and tau

In the present study, BACE1 activity correlated positively with tau concentrations in the AD group. This is a challenging observation at first glance, which is, nevertheless, in line with three previous individual reports [19,18,28] and a meta-analysis [18]. Tau is a marker of axonal degeneration, which may enhance BACE1 shedding, resulting in higher CSF levels and activity of BACE1 in AD [27]. Alternatively, as both tau and BACE1 are primarily located in neurons, their correlation may indicate that increased BACE1 activity in CSF is associated with protein release from decaying neurons.

4.2. BACE1 and markers of APP metabolism

We also found a positive correlation between BACE1 activity and sAPPβ levels. This association was expected because sAPPβ is a direct product of APP cleavage by BACE1 [29]. BACE1 activity did not correlate with the CSF levels of sAPPα in our study; this was also an expected result because there is no direct association between BACE1 and sAPPα, which is a product of α-cleavage rather than of β-cleavage [3]. In contrast to our findings, an association between sAPPα levels and BACE1 activity in CSF was observed in a previous study [19]. The authors argued that this surprising finding might be explained by the strong correlation between sAPPα and sAPPβ in CSF, suggesting tightly linked regulating processes, or alternatively by the fact that sAPPα may reflect overall APP levels. The expected correlation between BACE1 activity and $A\beta_{1-42}$ levels was probably obscured in our study by other factors
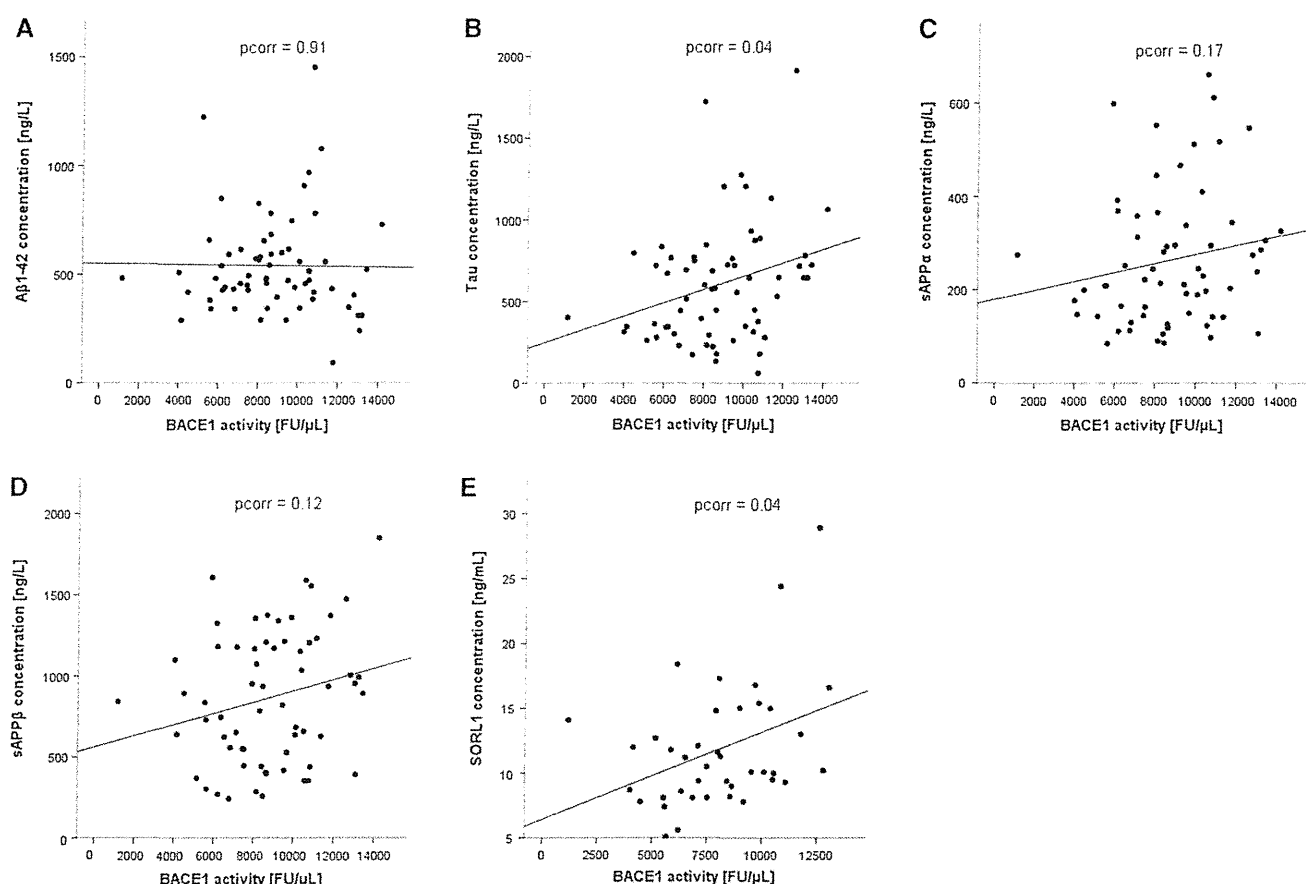
Fig. 1. Scatterplots showing the correlation between cerebrospinal fluid BACE1 activity and the concentrations of (A) $A\beta_{1-42}$, (B) tau, (C) sAPPα, (D) sAPPβ, and (E) SORL1 in the AD group.

influencing Aβ deposition in senile plaques, which is thought to be mirrored by decreased $A\beta_{1-42}$ concentrations in CSF.

4.3. BACE1 and SORL1

We also report a positive correlation between BACE1 activity and SORL1 concentrations in CSF. This finding is consistent with in vitro studies showing a direct interaction between BACE1 and SORL1 [30]. SORL1 levels were found to be reduced in the Golgi apparatus and early endosomal compartments in AD [31-33], allowing or promoting APP processing by BACE1 and α-secretase [30,34,35]. The ELISA used in our study determines the soluble form of SORL1, which consists of the extracellular domain of the membrane-spanning SORL1 protein [24]. This extracellular fragment seems to be less efficient than full-length SORL1 in mediating APP transport through the Golgi apparatus [30] because SORL1 fragments have altered binding capacities compared with the full-length SORL1 receptor [36,37]. Taking into account that AD pathology is associated with increased BACE1 activity, it can be hypothesized that the intracellular decline of full-length SORL1 levels in AD is caused by an elevation in the endoproteolytical cleavage of SORL1, resulting in increased concentrations of the less

efficient soluble SORL1 that we measure in CSF. However, it has to be mentioned that no causalities can be derived from a study reporting associations between CSF protein levels and that the validity of our argumentation will have to be tested in future studies.

4.4. Limitations

The present study should be viewed in light of a number of limitations. The size of the control group was relatively small, and the control subjects were younger than the patients with AD. As a consequence, we were not in a position to explore the differences in BACE1 activity between physiological aging and AD. Furthermore, although not very likely, some patients with causes for cognitive impairment other than AD might have been included despite the rigorous diagnostic assessment. No pathological verification of diagnoses was available, but current diagnostic criteria for AD have been shown to be very accurate for populations recruited at specialized centers. On the one hand, further research on larger samples with age-matched control groups is needed to replicate our findings; on the other hand, genetic variants of SORL1 will also have to be considered in future analyses. The thus-far inconclusive findings on SORL1 in CSF [16,17]

might probably be partly explained by the genetic association of four other vacuolar protein sorting 10 protein-domain receptors with sporadic AD [35], which may dilute the effect of any individual marker including SORL1.

5. Conclusion

Our study provides a further piece of evidence pointing to the associations between BACE1 on the one hand and relevant CSF markers of AD on the other hand, including the soluble form of the APP sorting receptor SORL1, the first product of APP cleavage by BACE1 (sAPPβ), and a marker of axonal degeneration (tau). Although our investigation was not designed to establish any diagnostic validity of the studied CSF proteins, it still strongly supports the relevance of BACE1 and SORL1 in CSF for AD and their potential benefit as AD biomarkers and therapeutic targets.

Acknowledgments

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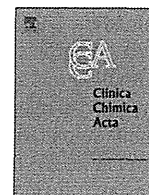
Increased circulating soluble LR11 in patients with acute coronary syndrome

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ARTICLE INFO

ABSTRACT

Background: LR11 (also so called SorLA or SORL1) is a novel marker of intimal smooth muscle cell (SMC) proliferation. Vascular SMCs play important roles in the development of atherosclerosis interacting with macrophages in a vulnerable plaque of patients with acute coronary syndrome (ACS).The present study determines whether soluble LR11 (sLR11) is associated with ACS.
Methods: We studied 100 patients with coronary artery disease (CAD) comprising 50 consecutive patients with acute coronary syndrome (ACS; mean age 62.3 ± 13.0 years; male 78.0%) who were successfully treated with percutaneous coronary intervention and 50 age- and sex-matched stable angina pectoris (SAP) patients as control. Concentration of sLR11 was measured by sandwich enzyme-linked immunosorbent assay method.
Results: Circulating sLR11 was significantly increased in patients with ACS compared with SAP (9.88 ± 2.78 vs. 8.18 ± 1.11 ng/ml, $p<0.01$). Multivariate logistic regression analysis indicated that sLR11 was independently associated with ACS (odds ratio (OR), sLR11 quartile increment, 2.18, 95% confidence interval (CI) 1.21–4.19, $p<0.01$). Among various biomarkers of acute coronary syndrome, hsCRP were significantly correlated with LR11 ($r=0.480$, $p<0.01$). *Conclusions:* There is a statistical significant association between LR11 and ACS and may be a useful biomarker for the development of acute coronary syndrome.

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1. Introduction

Acute coronary syndrome (ACS) remains a major cause of mortality and morbidity despite advances in cardiovascular therapies [1–3] and it is mainly characterized by the rupture of lipid-rich vulnerable atherosclerotic plaque with subsequent thrombus formation [4,5]. Many studies have investigated the pathological mechanisms of ACS and inflammation is recognized as a key step in the pathogenesis of acute thrombotic events [6,7]. A novel diagnostic biomarker derived from ruptured plaque would be useful but remains to be established.

LR11 (also so called SorLA or SORL1) is a member of the LDL receptor family, and is highly expressed in atheromatous plaques of animal experimental model, especially in intimal smooth muscle cell (SMC) but not in medial SMC [8,9]. The overexpression of LR11 protein enhances SMC migration via the activation of the urokinase-type plasminogen

activator receptor that regulates inflammatory monocyte adhesion [9,10]. We previously reported that circulating LR11 can be immunologically detected in serum using a novel sandwich enzyme-linked immunosorbent assay (ELISA) and specific monoclonal antibodies against human LR11 [11,12]. Circulating soluble LR11 levels positively correlate with the intima-media thickness in patients with dyslipidemia [11]. In addition, we also demonstrated increased levels of soluble LR11 in patients with stable coronary artery disease [13]. However, circulating LR11 levels in ACS have not been evaluated. The present study therefore evaluated the clinical significance of circulating LR11 in patients with ACS.

2. Materials and methods

2.1. Subjects

The present study is a cross-sectional case–control study. We enrolled 50 patients with ACS who were successfully treated with percutaneous coronary intervention (PCI) at Juntendo University Hospital between November 2008 and December 2009 and 50 age- and sex-matched patients with stable angina pectoris (SAP) as controls (mean age 62.4 ± 12.7 years; male, 78 %). Patients with previous coronary revascularization, malignant disease, inflammatory disease, and hemodialysis were excluded from the study. We defined ACS as acute myocardial

infarction (AMI) and unstable angina (UAP). AMI was defined as increased cardiac enzymes (troponin or MB fraction of creatine kinase) with ischemic symptoms and subclassified AMI as ST-segment elevation myocardial infarction (STEMI) and non ST-segment elevation myocardial infarction (NSTEMI) according to the presence or absence, respectively, of at least 0.1 mV ST-segment elevation in at least 2 contiguous leads. Unstable angina (UAP) was diagnosed based on the presence of ischemic symptoms with the ST–T change but without an increase in cardiac enzymes, or myocardial necrosis indicated as an increase in cardiac enzymes regardless of ST-segment change. The ACS group was divided into STEMI and UAP/NSTEMI subgroups. SAP was defined as effort angina with a stable profile of symptoms for at least 3 months before admission. Written informed consent was obtained from all patients to underego PCI using standard techniques. This study adhered to the Declaration of Helsinki and was approved by our institutional internal review board. The choice of stent type and device was left to the discretion of the operators at our cardiology center.

2.2. Blood samples

Arterial blood samples were collected from all patients before coronary angiography in the operating room. The samples were centrifuged at 1000×g for 10 min and serum samples were stored at −80 C. Soluble LR11 (sLR11) was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) method with our specific monoclonal antibodies directed against human LR11 that we previously established [12]. In brief, sLR11 in serum was immunologically identified as 250-kDa protein in serum fluid by SDS–PAGE separation, and was purified using a receptor-associated protein and monoclonal antibodies that bind to intact sLR11 without prior purification. The sLR11 immunoassay used a combination of anti-LR11 monoclonal antibodies (M3 and R14). Assay characteristics concerning sLR11 immunoassay, for example, the inter-, and intra-assay CVs, as well as the working range, and the mean back-fit value for the lowest standard giving acceptable precision, and the lower limit of detection has been described in our previous article [12]. Concentrations of serum high-sensitive C-reactive protein (hs-CRP) were measured using a validated immunoassay and an autoanalyzer. Levels of circulating Troponin T (TnT) and CD40 ligand (CD40L) were measured in patients with ACS. Serum cardiac troponin T was measured using a chemiluminescent enzyme immunoassay kit (Determiner CL TnT, Kyowa Medex, Tokyo, Japan). CD40L was quantified using human CD40L ELISA kit (R&D systems, Minneapolis, MN). Other markers were determined by routine laboratory methods.

2.3. Statistical analysis

Results are expressed as means ± SD or as ratios (%) and numbers for categorical data. The distribution of continuous variables was visually assessed from frequency histograms and using the Kolmogorov-Smirnov test. The hs-CRP and blood glucose on admission (BG on Ad) were skewed and thus we used natural log-transformed hs-CRP and BG on Ad. Continuous variables were compared using an unpaired t-test or Mann–Whitney U-test. Categorical variables (presented as frequencies) were compared using either chi-square test or Fisher's exact probability test. LR11 values across the three groups were compared using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Associations between measured parameters were evaluated using Spearman's rank correlation coefficient analysis. Predictive variables for ACS adjusted for potential confounding factors were identified by multiple logistic regression analysis. The effects of biomarkers including hs-CRP, blood glucose on admission and sLR11 in this model were evaluated as quartile increments in the concentration of each. The univariate model included the variables of age, gender, diabetes, hypertension, dyslipidemia, current smoking, use of statins, angiotensin converting enzyme and/or angiotensin receptor blockers, hs-CRP, blood glucose on admission (BG on Ad) and sLR11. Statistically significant

variables in the multivariable logistic regression analysis selected using stepwise forward selection were subsequently included in a new model. All data were statistically analyzed using JMP8.0 (SAS Institute Inc., Cary, NC) and SPSS v.18.0 (Chicago, IL). A p<0.05 was considered statistically significant.

3. Results

3.1. Characteristics of subjects

A comparison of the baseline characteristics between the ACS and SAP groups is shown in Table 1. Diabetes, metabolic syndrome, and hypertension were comparable between them, whereas current smoking and dyslipidemia were more frequent in the ACS and SAP group, respectively. The levels of total cholesterol, LDL-C, hs-CRP, WBC, and blood glucose on admission were significantly higher in the ACS group.

3.2. Levels of LR11

Levels of circulating soluble LR11 were significantly higher in patients with ACS than that with SAP (9.88 ± 2.78 vs. 8.18 ± 1.11 ng/ml, p<0.01). We also compared LR11 levels among 50 ACS patients subclassified as 32 patients represented STEMI and 18 diagnosed as NSTEMI/UAP, respectively. Levels of sLR11 were significantly higher in patients with STEMI compared with the other 2 groups (Fig. 1).

Table 1
Baseline Clinical Characteristics.

	SAP (n = 50)	ACS (n = 50)	P
Age	62.4 ± 12.5	62.3 ± 13.0	NS
Male Gender, n (%)	39 (78.0)	39 (78.0)	NS
Diabetes, n (%)	18 (36.0)	17 (34.0)	NS
Metabolic syndrome	30 (60.0)	23 (46.0)	NS
Hypertension, n (%)	38 (76.0)	33 (66.0)	NS
Dyslipidemia, n (%)	43 (86.0)	34 (68.0)	< 0.05
Current Smoker, n (%)	9 (18.5)	28 (56.0)	< 0.01
Family history, n (%)	15 (30.0)	13 (61.1)	NS
CKD, n (%)	9 (18.0)	11 (22.0)	NS
Angiographic degree of CAD			NS
1-vessel disease, n (%)	26 (52.0)	26 (52.0)	
2-vessel disease, n (%)	11 (22.0)	14 (28.0)	
3-vessel disease, n (%)	13 (26.0)	10 (20.0)	
LVEF, %	62.7 ± 10.3	62.8 ± 9.9	NS
ACE-I/ARB	31 (62.0)	13 (26.0)	<0.01
Statin	37 (74.0)	13 (26.0)	<0.01
BMI, kg/m²	24.2 ± 2.9	25.2 ± 3.5	NS
Waist, cm	88.2 ± 8.2	90.7 ± 8.2	NS
SBP, mmHg	146.3 ± 24.4	139.3 ± 24.0	NS
DBP, mmHg	77.5 ± 13.2	80.2 ± 17.9	NS
TC, mg/dl	171.1 ± 37.4	204.3 ± 38.5	<0.01
LDL-C, mg/dl	93.1 ± 30.2	131.3 ± 29.5	<0.01
HDL-C, mg/dl	49.3 ± 13.1	45.1 ± 11.1	0.09
TG, mg/dl	131.5 ± 52.8	145.7 ± 105.1	NS
FBG, mg/dl	103.5 ± 21.9	109.9 ± 28.2	NS
HbA1c, %	5.8 ± 1.1	6.2 ± 1.5	NS
BNP, ng/dl	56.3 ± 83.1	105.2 ± 209.4	NS
hs-CRP, mg/dl	0.25 ± 0.68	1.33 ± 2.67	<0.01
eGFR, ml/min/1.73 m²	70.9 ± 16.3	76.2 ± 26.1	NS
WBC, /μl	5784 ± 1562	9489 ± 3125	<0.01
BG on admission	120.0 ± 38.8	166.3 ± 74.5	<0.01
sLR11, ng/ml	8.18 ± 1.11	9.88 ± 2.78	<0.01

ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BG, blood glucose; BNP, brain natriuretic peptide; CAD, coronary artery disease; CKD, chronic kidney disease; DBP, diastolic blood pressure; FBG, fasting blood glucose; LAD, left anterior descending artery; LCX, left circumflex coronary artery; LVEF, left ventricular ejection fraction; RCA, right coronary artery; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.
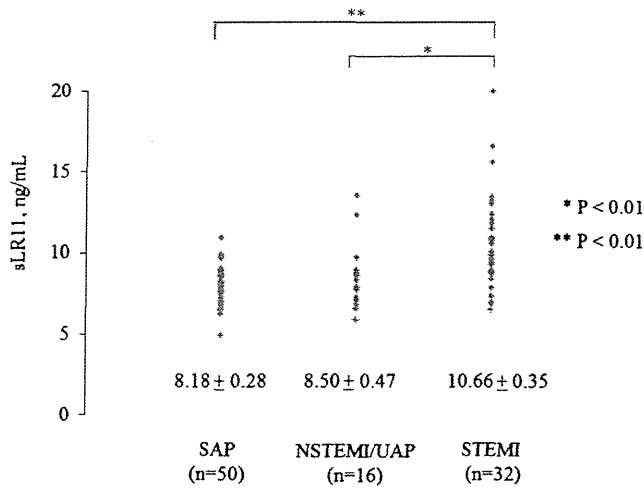
Fig. 1. Comparison of sLR11 among SAP, NSTEMI/UAP and STEMI. SAP, stable angina pectoris; NSTEMI/UAP, non ST-segment elevation myocardial infarction/unstable angina; STEMI, ST-segment elevation myocardial infarction.

3.3. Correlations between LR11 and clinical parameters

As shown in Table 2, serum LR11 levels positively correlated with BMI and LDL-C in all patients (r = 0.217, p < 0.05 and r = 0.304, p < 0.01, respectively). In ACS patients, LR11 positively correlated with hs-CRP (r = 0.480, p < 0.01, Fig.2A), WBC (r = 0.413, p < 0.05) and blood glucose on admission (r = 0.437, p < 0.01, Fig. 2B). LR11 tended to correlate swith TnT and CD40L but not significant (r = 0.231, p = 0.11 and r = 0.230, p = 0.11, respectively).

3.4. Multiple logistic regression analysis

We evaluated predictors of ACS for the entire study population using univariate and multivariate logistic regression analysis. The multivariate logistic regression analysis in Table 3 shows that sLR11 is independently associated with ACS after adjusting for confounding factors (odds ratio (OR), sLR11 quartile increment, 2.18, 95% CI 1.21–4.19, p < 0.01).

4. Discussion

The present findings demonstrated that circulating soluble LR11 levels is significantly higher in patients with ACS than with SAP. We also showed that sLR11 is significantly and positively correlated with hs-CRP. Multivariate analysis indicated that increased sLR11 could be an independent variable for ACS after adjustment.
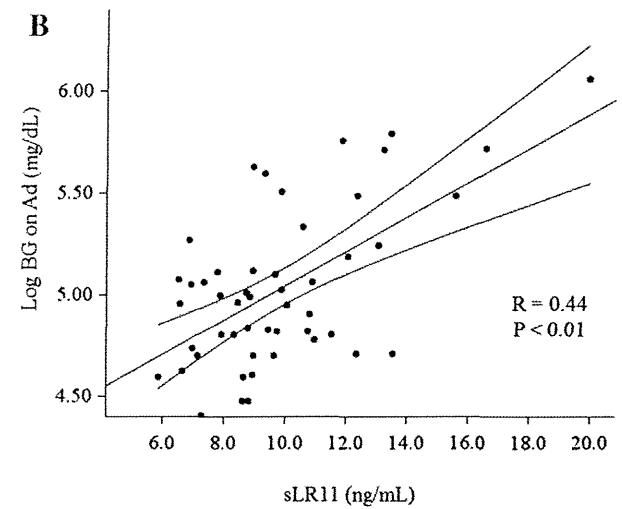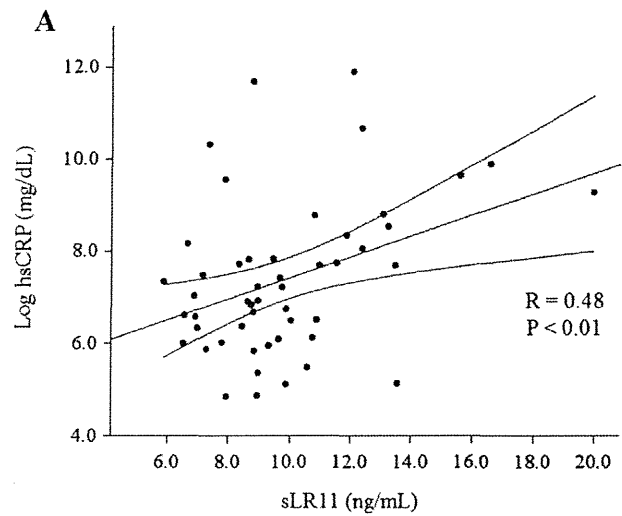

Fig. 2. Correlation between levels of sLR11 and various biomarkers in ACS. Fig. 2A, correlation between levels of sLR11 and hsCRP in ACS; Fig. 2B, correlation between levels of sLR11 and blood glucose on admission in ACS.

LR11, is a potential novel biomarker of vascular smooth muscle cells, that are abundantly expressed in the intimal SMC at the border between intima and media in plaque areas of apo E knockout mice [9]. Circulating LR11 positively correlates with intimal-media thickness in patients with dyslipidemia [11] and levels of soluble LR11 are higher in patients with coronary artery disease [13]. Our previous studies

Table 2
Correlations between LR11 and other parameters.

	r	P
Age	0.017	NS
BMI, kg/m^2	0.217	<0.05
SBP, mmHg	−0.029	NS
DBP, mmHg	0.130	NS
LDL-C, mg/dl	0.304	<0.01
HDL-C, mg/dl	−0.050	NS
TG, mg/dl	0.130	NS
BG, mg/dl	0.111	NS
eGFR, mL/min/1.73 m^2	−0.067	NS
HbA1c, %	0.020	NS
BNP, ng/mL	0.119	NS

BG on Ad, blood glucose on admission; DBP, diastolic blood pressure; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

Table 3
Univariate and multivariate logistic regression analysis model for prediction of ACS.

	Univariate			Multivaritate		
	OR	95%CI	P	OR	95%CI	P
Age, y	1.00	0.97–1.03	NS	1.04	0.99–1.10	NS
Gender, male	1.00	0.38–2.60	NS	Not selected		
Diabetes, yes	0.92	0.40–2.09	NS	0.24	0.04–0.91	<0.05
Hypertension, yes	0.61	0.25–1.46	NS	Not selected		
Dyslipidemia, yes	0.34	0.12–0.90	0.03	Not selected		
Current smoking, yes	5.80	2.40–15.1	<0.01	11.9	2.59–74.0	<0.01
Statin use, yes	0.13	0.05–0.30	<0.01	0.38	0.10–1.33	NS
ACEI/ARB use, yes	0.22	0.09–0.51	<0.01	0.36	0.09–1.31	NS
High-sLR11, QJ	1.92	1.32–2.88	<0.01	2.18	1.21–4.19	<0.01
High-hsCRP, QJ	2.48	1.65–3.90	<0.01	2.06	1.16–3.91	<0.05
High-BG on Ad, QI	2.18	1.47–3.32	<0.01	2.83	1.52–5.83	<0.01

ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BG on Ad, blood glucose on admission; QJ, quartile increment.

implied that circulating LR11 reflects the pathological status of vascular smooth muscle cells in atherosclerotic lesions. Vascular smooth muscle cells play important roles in the development of atherosclerosis. Medial SMC migrate into the subendothelial space, proliferate, and produce extracellular matrix to form atheromatous plaques in response to inflammatory cytokines. The precursor of ruptured culprit lesions in patients with ACS is a thin cap fibroatheroma, characterized by a necrotic core and an overlying thin cap that lacks an extracellular collagen matrix [7,14]. The present study found significantly higher levels of soluble LR11 in patients with ACS. The pathological roles of LR11 in vulnerable plaque remain uncertain, but several possible explanations can be considered. Macrophages and vascular smooth muscle cells promote the local release of matrix metalloproteinases (MMP) that degrade supportive collagen, resulting in fibrous cap breakdown and enhanced plaque vulnerability [15–17]. The phenotypic modulation of smooth muscle cells influences the production of matrix-degenerating enzymes, monocyte recruitment and the expression of pro-inflammatory cytokines [18] and LR11 is expressed in synthetic intimal smooth muscle cells [19] that produce higher levels of matrix-degrading proteases resulting in thinning of the fibrous cap.

LR11 also significantly and positively correlated with hs-CRP, which plays an important role as an inflammatory mediator. Inflammation drives the formation and progression of atherosclerotic plaque and CRP is predominantly produced in the liver during the acute phase, but it is also expressed in smooth muscle cells within atherosclerotic lesions [20], where it is implicated in various aspects of atherogenesis and plaque vulnerability. We reported that soluble LR11 also regulates adhesion, migration and lipid accumulation in macrophages through urokinase-type plasminogen activator receptors [21]. We also demonstrated that sLR11 enhances scavenger receptor expression that contributes to foam cell formation in atherogenesis in vivo. These results imply that LR11 is a potential regulator of vulnerable plaque formation.

Soluble LR11 enhances the expression of urokinase-type plasminogen activator (uPAR) in macrophages and its expression is increased in the circulating monocytes of patients with AMI [22]. Urokinase-type plasminogen activator implicated in a broad spectrum of pathophysiological processes, including inflammation, fibrinolysis, proteolysis, atherogenesis and plaque destabilization, all of which contribute to the pathogenesis of myocardial infarction [23,24]. Cozen AE, et al. reported that macrophage-targeted overexpression of uPAR causes accelerated atherosclerosis, coronary artery occlusion, and premature death in apo E knockout mice [25]. Another study demonstrated that high levels of uPAR expression in vulnerable carotid plaque with high macrophage density and a ruptured fibrous cap [26]. These findings suggest that levels of soluble LR11 in ACS that are elevated via uPAR activation are associated with these pathophysiological conditions.

The present study has several limitations. Firstly, this is a single center study with a small patient cohort and thus unknown confounding factors might have affected the results regardless of the adjusted analysis. Further studies with a larger sample size are needed to confirm the results and to validate the clinical implication of LR11 as a diagnostic biomarker of ACS. Secondly, the limitations inherent in any cross-sectional study that a single sample at a specific time point might not reflect the natural course of a disease must be considered. The relationship between LR11 and other biomarkers at serial time points after patients with ACS are admitted to the hospital require further validation. Thirdly, several experimental studies have found that statin and angiotensin II type1 receptor blockers inhibit LR11 expression in SMCs [11,19]. More patients with the SAP group received statins and ARBs than the ACS group in the present study. This is because stable angina pectoris patients had been medically treated on the basis of current guideline, in contrast, most of patients with ACS admitted to our hospital for the first time without any medical background. These drugs might have attenuated circulating LR11 levels. In conclusion, there is a

statistical significant association between LR11 and ACS. We believe that LR11 might serve as new biomarker reflecting different aspects of atherosclerosis, however the mechanism of how sLR11 plays a role in the pathophysiological conditions of ACS requires elucidation.

Acknowledgments

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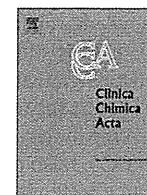
Circulating soluble LR11/SorLA levels are highly increased and ameliorated by chemotherapy in acute leukemias

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ARTICLE INFO

ABSTRACT

Background: LR11/SorLA, a receptor interacting with CD87 on monocytes and macrophages, is highly expressed on human immature hematopoietic stem cells. However, it is unknown whether LR11 is expressed on premature leukemic cells, and whether the levels of circulating soluble LR11 (sLR11) shed from leukemic cells correlate with disease state.
Methods: The expression of LR11 on leucocytes and leukemic cells was examined by flow cytometry. Serum sLR11 levels were measured by ELISA in patients with various hematological diseases, including 43 acute myeloid leukemia (AML) and 23 acute lymphoblastic leukemia (ALL) patients. Data were subjected to statistical analysis for validation of sLR11 levels and patients' clinical data.
Results: LR11 is specifically expressed in monocytes, and surface levels on leukemic cells are highly induced in both AML and ALL. sLR11 levels of acute leukemia patients were significantly increased (P<0.001) (ALL, 73.5 ± 93.5 ng/ml; AML, 26.8 ± 29.1 ng/ml) in comparison to controls (9.2 ± 3.3 ng/ml). Patients with AML and ALL in remission showed significantly decreased sLR11 levels to below 20 ng/ml.
Conclusions: LR11 and its released soluble form are strongly elevated in acute leukemias. Remarkably, this increase in circulating sLR11 levels is ameliorated at complete remission.

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1. Introduction

Chemotherapy in combination with the use of allogeneic hematopoietic stem cell transplantation has improved the prognosis of acute leukemia patients [1–3]. However, primary induction failure and relapse are still major problems affecting survival, requiring efficient prognostic factors at diagnosis and for monitoring minimal residual disease (MRD). Migration through vascular endothelia and underlying extracellular matrices is essential for mobilization and homing processes of hematopoietic stem and progenitor cells (HSPC) between bone marrow and circulation [4]. Understanding the underlying regulatory features is pivotal for evaluating the (i) expansion of leukemic cells originating from post-chemotherapy bone marrow MRD and (ii) efficacy of HSPC mobilization induced by G-CSF and analogues for transplantation in patients with acute leukemias [5,6]. HSPC migration is strictly controlled by a close interplay between chemokines and adhesion molecules selectively expressed in these migrating cells, stromal cells, or endothelial cells [4–6]. Recent clinical studies suggest that the levels of circulating soluble forms of adhesion molecules and/or cell surface receptors are associated with G-CSF-induced mobilization of HSPC and with the prognosis of leukemia patients under chemotherapy [7,8]. However, our

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