Overexpression of an isoform of *AML1* in acute leukemia and its potential role in leukemogenesis

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Running title: *AML1* isoform induces leukemia

Abstract

AML1/RUNX1 is a critical transcription factor in hematopoietic cell differentiation and proliferation. From the *AML1* gene, at least three isoforms, *AML1a*, *AML1b* and *AML1c*, are produced through alternative splicing. AML1a interferes with the function of AML1b/1c, which are often called AML1. In the current study, we found a higher expression level of *AML1a* in ALL patients in comparison to the controls. Additionally, AML1a represses transcription from promotor of macrophage-colony simulating factor receptor (M-CSFR) mediated by AML1b, indicating that AML1a antagonized the effect of AML1b. In order to investigate the role of *AML1a* in hematopoiesis and leukemogenesis *in vivo*, bone marrow mononuclear cells (BMMNCs) from mice were transduced with AML1a and transplanted into lethally irradiated mice, which develop lymphoblastic leukemia after transplantation. Taken together, these results indicate that overexpression of AML1a may be an important contributing factor to leukemogenesis.

Key words: AML1; isoform; M-CSFR; RT-PCR; transactivation; leukemia

Introduction

AML1, also known as RUNX1, PEBP2αB, or CBFα2, is a transcription factor that plays a crucial role in the proliferation and differentiation of hematopoietic cells. AML1 has a DNA binding domain, known as the Runt homology domain (RHD), and a transactivation domain that binds to and regulates target genes respectively (Miyoshi *et al*., 1995). *AML1* are affected by chromosomal translocations found in human leukemia, including t(8;21), t(3;21) and t(12;21) (Miyoshi *et al*., 1991, Nucifora, *et al*, 1993, Golub *et al*, 1995). The t(8;21) is most frequent chromosomal translocation in acute myeloid leukemia, and it joins AML1 to ETO, resulting in the formation of AML1/ETO fusion gene in which AML1 retains RHD, but lacks the transactivation domain (Meyers *et al*., 1995; Tanaka *et al*., 1995). The lack of transactivational domain is supposed to be the event that triggers leukemogenesis (Tanaka *et al*., 1995).

AML1 is essential for hematopoiesis in early development as well as in adulthood. *AML1*-null embryos die typically around E12.5 because of a lack of fetal liver hematopoiesis and hemorrhage in the central nervous system (Okuda *et al*., 1996, Wang *et al*., 1996). Loss of *AML1* function in adulthood leads to a number of disturbance in hematopoiesis, including retarded megakaryocytic maturation and impaired T and B-lymphocytic differentiation (Ichikawa, *et al*., 2004). In addition, it has been proved that *AML1*-deficient cells are susceptible to malignant transformation (Kundu *et al*., 2005).

AML1 regulates promoters or enhancers of many target genes, including

interleukin 3 (IL-3) (Uchida H, *et al*.,1997), myeloperoxidase (Britos-Bray *et al*., 1997), neutrophil elastase (Nuchprayoon, *et al*.,1994), granulocyte-macrophage CSF (GM-CSF) (Cockerill *et al*., 1996), macrophage colony-stimulating factor receptor (M-CSFR) (Rhoades, et al.,1996; Zhang et al.,1996) and T-cell antigen receptor subunits (TCRs) (Fujii *et al*., 1998, Meyers *et al*., 1995). The RHD is near the N-terminus and contains approximately 128 amino acid residues (Daga *et al*., 1992, Kagoshima *et al*., 1993). Affinity of AML1 for its target DNA sequences increases significantly upon heterodimerization with CBFβ (Meyers *et al*., 1993; Wang *et al*., 1993; Ogawa *et al*., 1993a).

At least three alternative splice variants of the *AML1* gene (*AML1a*, *AML1b* and *AML1c*) have been identified to date (Miyoshi *et al*., 1995). The proteins encoded by *AML1b* and *AML1c* have a RHD in the N-terminus and a transactivation domain in the C-terminus (Miyoshi *et al*., 1995). In contrast, the protein encoded by *AML1a* has a RHD but lacks the transactivation domain. As such, the function of AML1 is believed to be mediated by AML1b and AML1c which are considered to have the same function (Miyoshi *et al*., 1995). Experiments using transient transfection have demonstrated that AML1b, but not AML1a, transactivated TCRs (Meyers, *et al*., 1995; Fujii *et al*., 1998) and GM-CSF (Frank *et al*., 1995). AML1a has no transactivational function by itself, but inhibits the transcriptional activity of AML1b by competing for the DNA sequence of target genes with a higher affinity (Tanaka *et al*., 1995). Overexpression of AML1a inhibits the myeloid terminal differentiation of the myeloid precursor lineage 32Dcl3 induced by G-CSF. Recent evidence showed that

the overexpressed AML1a is higher in patients with AML than the normal controls (Tanaka *et al*., 1995). Accordingly, we hypothesize that AML1a, similar to the leukemia-associated fusion proteins, perturbs the normal function of AML1, and maybe contribute to leukemogenesis.

In our current study, we first examined the expression level of *AML1a* in BMMNCs of acute leukemia patients. Results indicated that *AML1a* expression level in ALL patients was higher than that in healthy donors. We further analyze the effects of *AML1a* and *AML1b* on the transactivity by using a luciferase reporter plasmid containing macrophage colony-stimulating factor receptor (M-CSFR) promoter. Result demonstrated that *AML1a* competes with *AML1b* to inhibit the transcription of M-CSFR. Finally the *in vivo* effect of *AML1a* was explored by transplantation of AML1a expressing BMMNCs into lethally irradiated mice. Nine of 12 mice developed lymphoid leukemia between 16 and 45 weeks after transplantation. These results demonstrated: 1) *AML1a* is a functional AML1 “antagonist”; 2) overexpression of *AML1a* may serve as a critical oncogenic event to promote leukemogenesis.

Results

Expression of AML1 isoforms in acute leukemia (AL)

Bone marrow (BM) samples from 77 *de novo* AL patients (AML 51, ALL 21, HAL 5) and 7 healthy donors were analyzed by semi-quantitative reverse transcriptase-PCR (RT-PCR). The characteristics of 77 de novo AL patients including median age, gender, FAB subtypes are summarized in Table 1. AML1a expression in AL cells is not associated with gender, age, initial white blood cell count and median percentage of BM blast cells. Expression level of *AML1a* in 21 ALL patients was significantly higher than that of the healthy controls (p=0.048, Figure 1). However, the expression of *AML1a* in the healthy controls did not differ significantly from that in AML or HAL (Figure 1). The data suggest that overexpression of AML1a might play an important role in leukemogenesis.

Effects of AML1 transcripts on the transactivation of M-CSFR gene

Because AML1 is a transcription factor and AML1a is over-expressed in ALL, next we analyzed the effects of AML1a on transcription of AML1 target gene. The CV-1 cells were transfected with a plasmid expressing *AML1a* or *AML1b*. At 36 h after transfection, activity of the luciferase reporter gene was analyzed by luminometer. As shown in Figure 2, the transcriptional activity of M-CSFR promoter was activated by *AML1b* (Figure 2a) in a dose-dependant manner, but not by *AML1a* (Figure 2b). A 3.8-fold increase in luciferase activity was observed in cells transfected with pCMV5-*AML1b* at a dose of 0.2μg, but not in cells transfected with

pcDNA3-FLAG-*AML1a* at the same dose (Figure 2a and b). The difference between the two isoforms was statistically significant ($P<0.01$). As shown in Figure 2c, transactivation of M-CSFR mediated by *AML1b* was abrogated by co-transfection with *AML1a* in a dose-dependent manner. The aforementioned effects were completely absent in cells with mutant M-CSFR promoter in which AML1 binding sequence was disrupted (Figure 2d), indicating the specificity of the findings.

AML1a induces the development of lymphoblastic leukemia.

Since AML1a could interfere the transcription regulation mediated by full length AML1, AML1b. Over-expression of AML1a in hematopoietic cells may result in impairment of hemapoiesis or development of leukemia. In order to address this issue, we analyzed the effects of AML1a in vivo. BMMNCs infected with the retroviral vector MSCV expressing a FLAG-AML1a fusion protein and a yellow fluorescent protein (YFP) (Figure 3a) were inoculated into the lethally irradiated female C57 BL/6J mice. BMMNCs infected with the vector expressing YFP only was used as control. Number of transplanted YFP positive cells in both groups was approximately 30,000 per mouse. The expression of the fusion proteins was confirmed by Western blot (data not shown). Number of YFP-positive cells in the peripheral blood (PB) as well as general health condition of the mice was surveyed closely. Nine out of total 12 mice in the AML1a group developed leukemia at 3-11 months (Figure 3b, Table 2). Signs of cachexia, such as loss of body weight in these mice coincided with a rapid rise of YFP-positive cells from an initial level of 2-3% to 10-25% in peripheral blood.

The median survival time of the mice in the AML1a group was 258 days. Autopsy of these mice revealed splenomegaly and hepatomegaly (Figure 3c and d), sometimes with thymoma and lymph node enlargement. BM, spleen, lung, liver, kidney and thymus were infiltrated with leukemia cells (Figure 3e, f and j).

Integration of *AML1a* in the genome of leukemic spleen cells was detected by genomic PCR (Figure 4a). RT-PCR was performed to analyze the expression of AML1a in spleen cells of mice (Figure 4b and c). The 293T cells transfected with plasmid pMSCV-FLAG-AML1a-IRES-YFP was used as a positive control. As indicated in Figure 4d, the FLAG-AML1a fusion protein was clearly detected in the spleen sample of the AML1a leukemic mice.

Flow cytometry showed that 30-60% of the BMMNCs from *AML1a* group were positive for YFP, in comparison to 8-15% in the control group. A further analysis revealed that there are two different phenotypes of T-lymphoblastic leukemia in mice with leukemia (Table 1). One was Sca-1^+, cytoplasmic $CD3^+$ ($cCD3^+$) (Figure 5a), the other was $CD3^+CD4^+CD8^+$ (Figure 5b).

To assess transplantability of the leukemia, spleen cells from each leukemic mouse were inoculated into four naive mice through the tail vein. All mice rapidly developed lymphoblastic leukemia, with an average latency of 47 days (data not shown).

Discussion

In the current study, we found that AML1a is overexpressed in patients of acute lymphoblastic leukemia. A previous study using a qualitative assay for *AML1a* mRNA (Tanaka *et al*., 1995) showed that *AML1a* could be detected in half of the patients with AML, whereas almost no expression of *AML1a* could be detected in normal subjects. Here, we used a semi-quantitative assay to expand the investigation in more subjects. Surprisingly, we did not find a significant difference in the expression of *AML1a* between AML patients and the healthy donors. Instead, a significant difference was found between ALL patients and the control.

AML1-ETO, as a dominant negative protein, blocks transactivation of the GM-CSF promoter by *AML1b* (Frank *et al*., 1995). AML1-ETO constitutes the first hit by blocking the differentiation of hematopoietic stem cell (HSC). Myeloid leukemia develops when the second hit occurs (Fenske *et al*., 2004). Initially, we suppose that, similar to AML1-ETO, AML1a lacks transcriptional activity, but binds to target genes with higher affinity than AML1b (Tanaka *et al*., 1995), and thus may contribute to leukemogenesis. It would influence myeloid differentiation and play a certain role in development of leukemia, so we choose *M-CSFR* as the target gene of AML1 to study. In our experiments, activity of the M-CSFR promoter was dose-dependently transactivated by *AML1b*, but not by *AML1a*. More importantly, *AML1a* interfered with the transactivational effect of *AML1b* in a dose-dependent manner. A previous study also demonstrated that such an inhibitory effect by *AML1a* could be reversed by the overexpression of *AML1b* suggesting a competitive mechanism.

In our experiments *in vivo*, 75% of the receipt mice after transplantation of BMMNCs that were infected with retrovirus containing AML1a developed leukemia. In line with the results in patients (higher expression of AML1a in ALL but not AML in comparison to healthy controls), the leukemia cells in these mice were lymphoid rather than myeloid. These findings were puzzling at the first glance. However, evidence has suggested that AML1 is indeed involved in the regulation of T-cell-specific gene expression (Ogawa *et al*., 1993b). In addition, the investigation of the AML1 transcription suggests that AML1 may be critically involved in differentiation of lymphoid precursors in adult hematopoiesis (Bäsecke *et al*., 2002). Moreover, transition of the T cells from the $CD4^-CD8^-$ (DN) to the $CD4^+CD8^+$ (DP) phenotype is impaired in transgenic mice bearing a truncated, dominant interfering form of AML1 (Runt) (Sato *et al*., 2003). Consistent with the findings by Sato *et al*., we found that over-expression of AML1a may also influence DN or DP phase of T cells. Finally, AML1 deficiency has been reported to predispose mice to T-lymphoblastic lymphoma (Kundu *et al*., 2005).

Our data suggest that *AML1a* over-expression may be a critical event in the development of leukemia. However, the over-expression of AML1a alone may not be sufficient for leukemogenesis. First, not all mice carrying BMCs-AML1a developed leukemia. Second, leukemia developed after a relatively long period, suggesting that additional mutations or hits may be required. Two different immuno-phenotypes of lymphoid leukemia were observed in leukemic mice, indicating that some mutations occurred in different development and differentiation stages of HSC. A recent study

by Tsuzuki *et al*. suggested that AML1a may enhance the self-renewal capacity of HSC. Based on the findings that the over-expression of AML1a increases the engraftment potential of hematopoietic stem and progenitor cells, it is suggested that AML1a may be applied in hematopoietic cell transplantation to expand the number of cells (Tsuzuki, *et al*., 2007). However, results from the current study provided experimental evidence that the safety to utilize AML1a in transplantation was called in question before a thorough investigation.

In conclusion, our study indicated that AML1a may play a critical role in leukemogenesis, especially in the development of lymphoid leukemia. In addition, lymphoblastic leukemia model established in this study may serve as a valuable tool for future studies.

Materials and Methods

Patients and cell lines

77 patients with *de novo* AL and 11 patients with AL in complete remission (CR) from our hospital and 7 healthy donors were studied after giving informed consent. Diagnosis and classification of the leukemia were made on the basis of Morphology-Immunology-Cytogenetics-Molecular biology (MICM) typing standard using the French-American-British system (Bennett *et al*., 1976, Lo Coco *et al*., 1995). CV-1, NIH 3T3 and 293T cell lines were grown in Dulbecco modified Eagle medium supplemented with 10% heat-inactivated fetal bovine serum.

RNA and DNA isolation, *RT-PCR, PCR and construction of plasmids*

See Supplementary Materials and methods.

Transient luciferase assay

CV-1 cells were seeded in six-well plates at a density of 5×10^5 per well. Transfection was performed by using calcium phosphate-mediated precipitation when cells reached 70% confluence. The following combinations of plasmids were used: 1.2 μg of pM-CSF-R-luc reporter plasmid or pM-CSF-R(mB)-luc reporter plasmid with different dosage of pcDNA3-Flag-*AML1a* and pCMV5-*AML1b* and with 1 μg of β-galactosidae plasmids. At 6 h after transfection, the medium was changed. The cells were harvested at 36 h post transfection for assays of β-galactosidase and luciferase activity (Promega; Madison, WI, USA).

Viral production, transduction and transplantation of murine bone marrow and tumor cell transplantation

293T cells were transfected with pMSCV-IRES-YFP, pMSCV-Flag-*AML1a*- IRES-YFP, the envelope-encoding plasmid pECO, or the packaging plasmid pGP using a method of calcium phosphate preciptation. Culture medium was collected at 48 and 72 h after the transfection, and filterd with 0.45 μm filters. Retrovirus titers were determined by transducing NIH3T3 cells (1×10^5) with serial dilutions of the retrovirus in the presence of 8 μg ml^{-1} polybrene (Sigma, Deisenhofen, Germany) at 72 h after infection. The titer was calculated by multiplication of the total number of

YFP-positive cells with the dilution facor of the retroviral supernatant. At 48 h post transduction, percentage of infected cells was determined by flow cytometric analysis of YFP expression. Bone marrow cells were harvested from male C57BL/6J donor mice 3 days after injection of 150 mg kg^{-1} 5-fluorouracil (5-Fu) (Sigma), and pre-stimulated overnight in Iscove modified Dulbecco medium (IMDM)/20% FCS supplemented with 10 ng ml^{-1} murine IL-3 (mIL-3), 10 ng ml^{-1} mIL-6 and 50 ng ml^{-1} mSCF. All cytokines were purchased from R&D system. Cells were transduced by 3 rounds spin infection (1200g, 25°C, 90 minutes) every 24 hours in retroviral supernatant supplemented with growth factors and 8 μg ml^{-1} polybrene. Cells were re-suspended in Hanks balanced salt solution and injected into the tail vein of lethally irradiated (9Gy) female C57BL/6J mice. Rate of YFP-positive cells in PB was closely monitored by FACS. 5×10^{6} spleen cell suspension of each leukemic mouse was administered to the recipient mice after an irradiation dose of 4.5 Gy. All animals were maintained in a special caging system with autoclaved food and water.

Western blot analysis

We performed protein lysate preparation and western blotting as previously described (Zhang *et al*, 2007). Anti-FLAG monoclonal antibody and anti-β-actin antibody was purchased from Sigma. The blots were visualized by chemiluminescence (ECL, Amersham, Freiburg, Germany).

Flow cytometry of mouse cells

For lineage marker analysis, cells (1×10^6) were incubated with monoclonal antibodies against Sca-1, c-Kit, Gr-1, Mac-1, Ter119, B220, CD19, Thy1.2, CD3, CD4, CD8, or their isotype controls (Biolegend，USA). The cells were then washed and applied for analysis on a FACSCalibur flow cytometer (Becton Dickinson San Jose, CA, USA).

Hematological and histological analysis

PB smears and BM cytospin slides were stained with Wright–Giemsa staining solution. Tissue samples were fixed with 10% phosphate-buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E) and observed under a light microscope.

Statistical methods

For statistical analysis, survival curves were produced using the Kaplan-Meier estimates, group distributions were compared parametrically using the student's t test and group distributions were compared non-parametrically using the Mann-Whitney U test or Chi-square test.

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pCMV5-AML1b plasmid, respectively. This work was supported by Major State Basic Research Development Program (2006CB910406) of China.

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Figure 1 Expression of *AML1a* gene in BMMNC of patients with ALL, AML, HAL and healthy donors. There was a significant difference between ALL patients and healthy donors. "–" shows median value;*, P<0.05; N.S.: not significant.

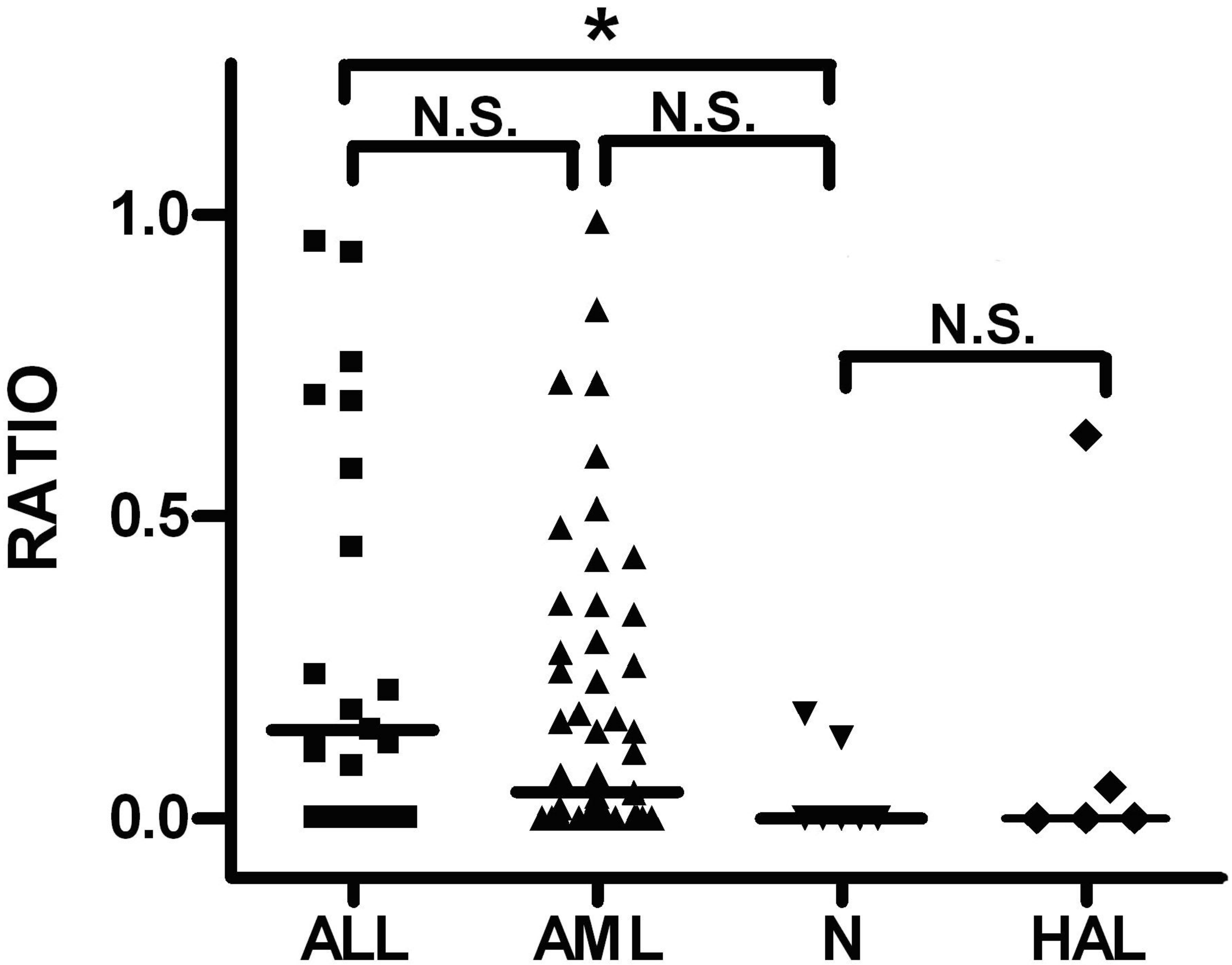
Figure 2 Effects of *AML1* transcripts on the transactivation of M-CSFR gene promoter. (a) Transactivity of *AML1b* alone at different dosages. (b) Transactivity of *AML1a* alone at different dosages. (c) Abrogation of *AML1b* transactivity by *AML1a* in a dose-dependent manner. (d) Effect of *AML1b* binding site mutation of M-CSFR promoter on transactivity. Error bars: standard error of the mean.

Figure 3 Gross and histopathology of AML1a mice with leukemia phenotype. (a) The structure of retroviral plasmids: pMSCV-IRES-YFP and pMSCV-FLAG-AML1a-IRES-YFP. (b) Kaplan-Meier survival curves (leukemia-free) of receipt mice transduced with either AML1a (n=12) or a vector control (n=12). Autopsy of these animals revealed: spleenomaly (c) (the upper panel is the control spleen), hepatomegaly (d) (the right panel is the control liver). Wright-Giemsa-stained PB (e) and BM (f) cytospin from representative leukemic AML1a mice (1000×) and hematoxylin and eosin (H&E) staining of involved organs (g-i) (400×). The normal framework was destroyed and infiltrated by large number of tumor cells in BM (g), spleen (h) and thymus (i).

Figure 4 Integration and expression of *AML1a* in spleen cells of leukemic mice. (a) Genomic DNA specific for AML1a in isolated leukemia cells as detected by genomic PCR. As a positive

control, a plasmid containing AML1a template was amplified. (b) RNA was isolated from the spleen. Cells were transduced with either AML1a or YFP only. (c) Expression of β-actin. M: marker. (d) Western blot analysis of FLAG (AML1a) protein from the spleen of the mice. 293T cells transduced with AML1a served as a positive control. SP cells in mouse # 21 transduced with YFP only were included as a negative control.

Figure 5 Flow-cytometric analysis of mononuclear cells from BM and spleen of AML1a mice with leukemia phenotype. (a) Increased Sca-1^{+}/cCD3^{+} cell populations in the YFP^{+} cells of BM and SP from a representative AML1a leukemic mouse (#105) phenotype as compared to those in the YFP+ cells of BM and SP from a control animal. (b) Flow cytometry analysis on BMCs and SP cells freshly isolated from an AML1a leukemia mouse (#109) with a T-lymphoblasticleukemia phenotype, and a control mouse. The plots showed expression of lineage-specific antigens (Sca-1, c-Kit, Thy1.2, CD3, CD4, CD8) versus YFP. Numbers indicated the percentage of cells in the quadrant.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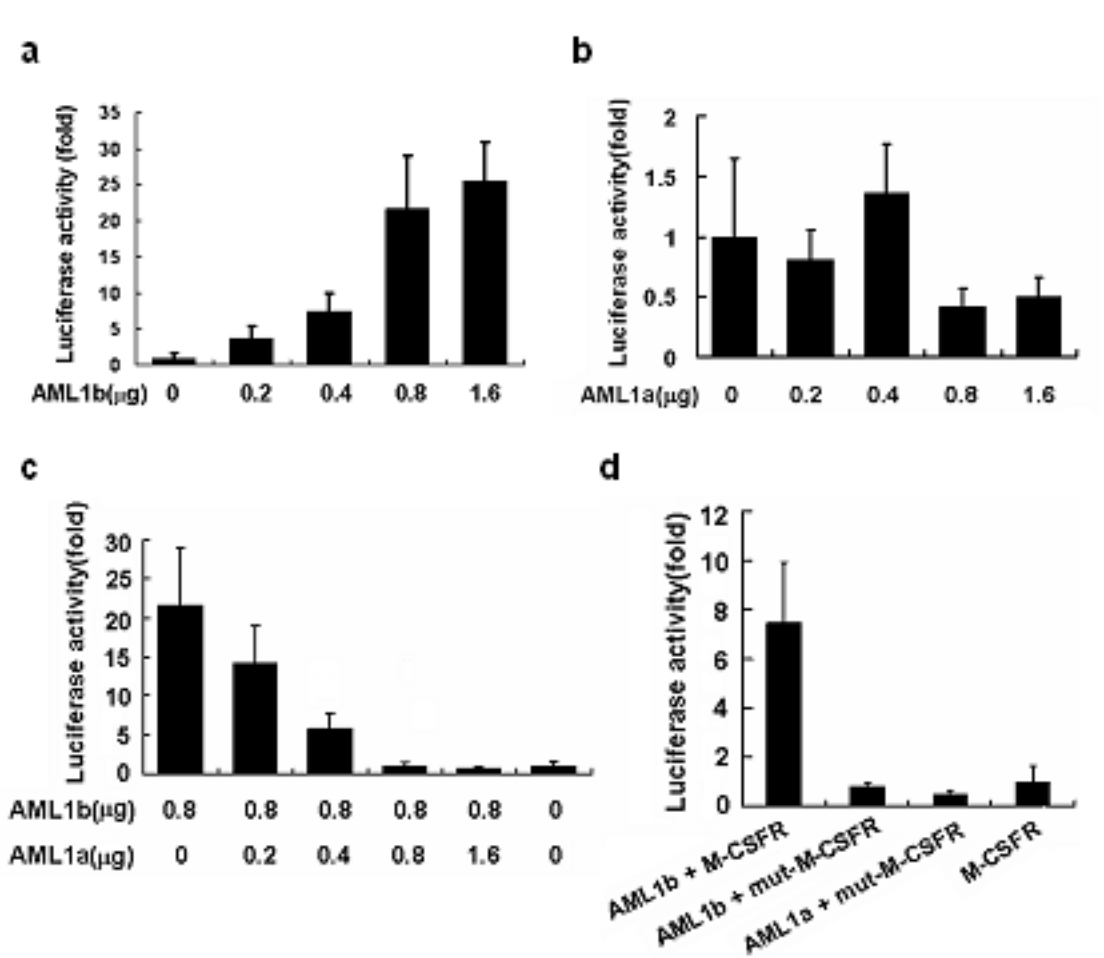
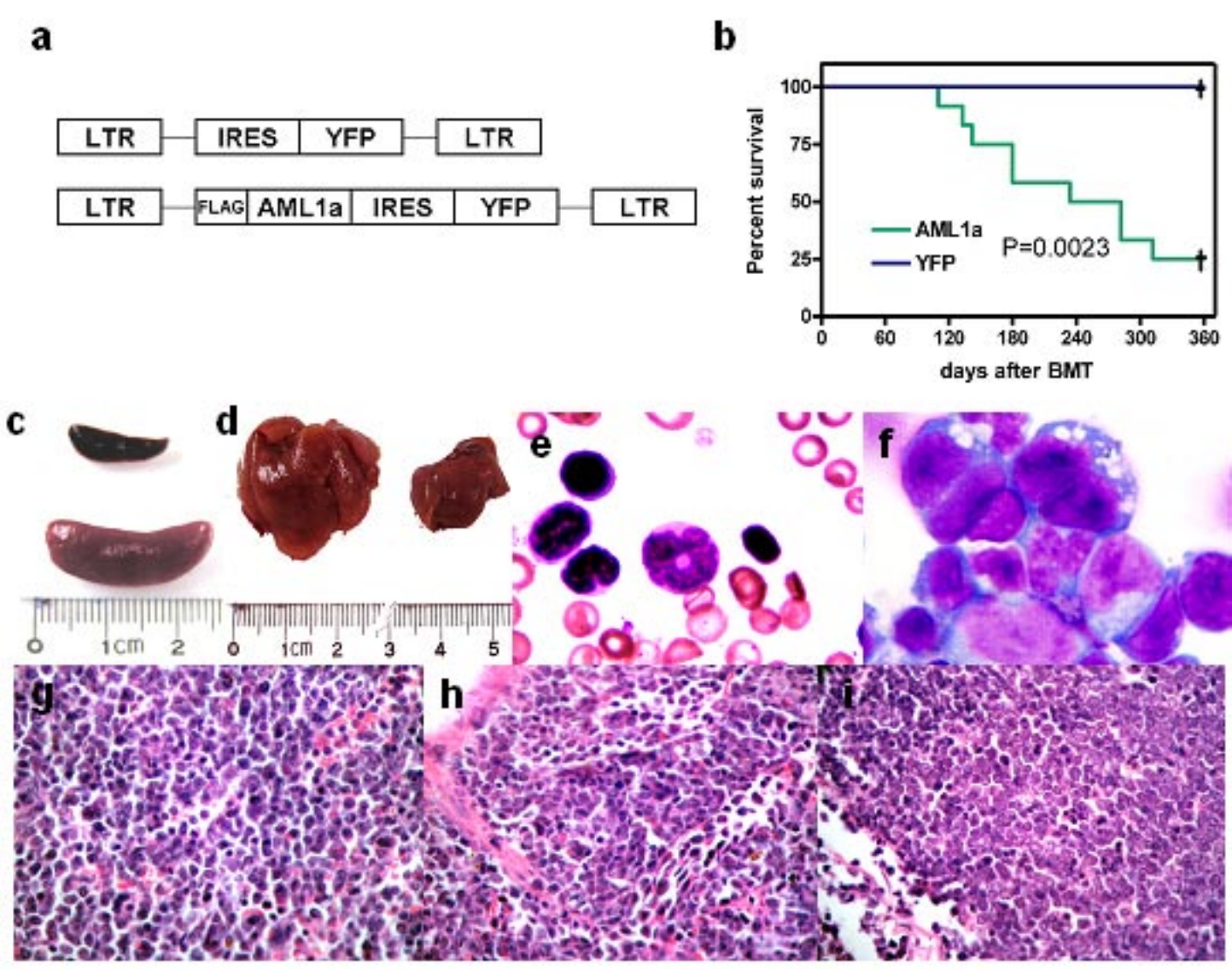
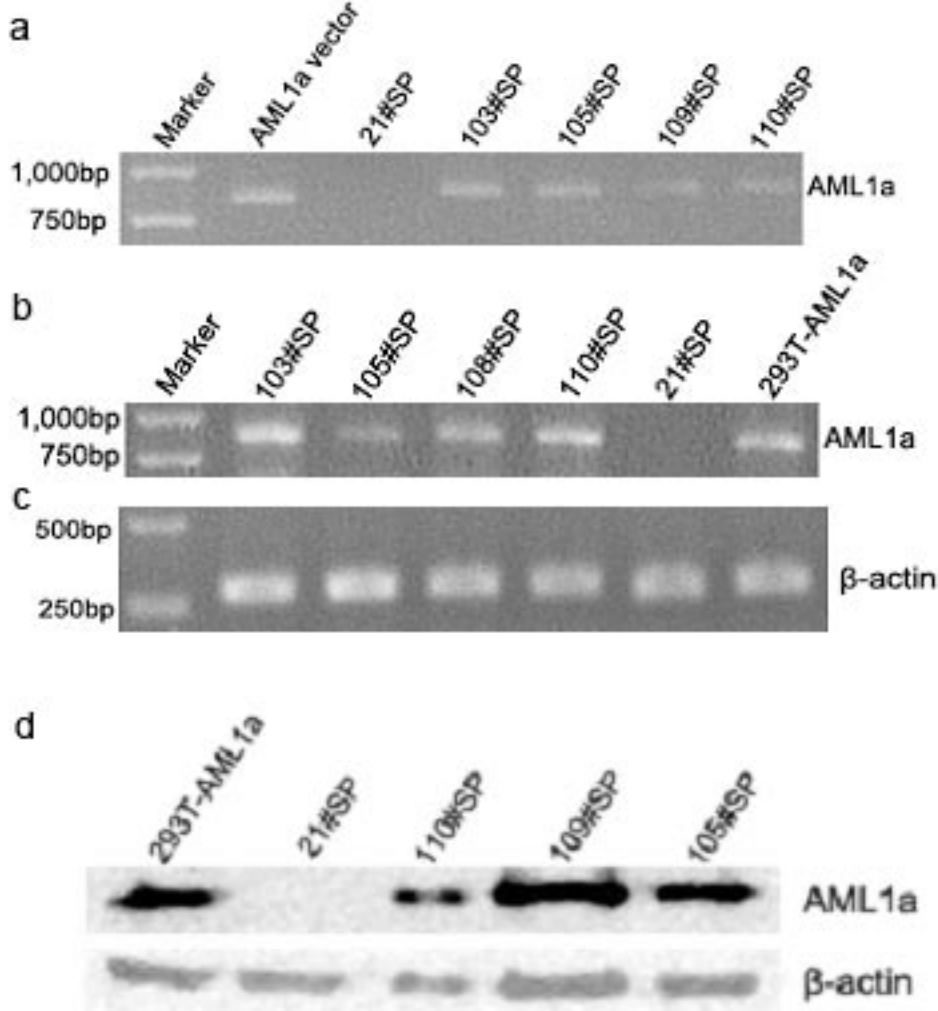
N
HAL


a
Luciferase activity (fold)
35
30
25
20
15
10
5
0
AML1b(μg) 0 0.2 0.4 0.8 1.6
b
Luciferase activity(fold)
2
1.5
1
0.5
0
AML1a(μg) 0 0.2 0.4 0.8 1.6
c
Luciferase activity(fold)
30
25
20
15
10
5
0
AML1b(μg) 0.8 0.8 0.8 0.8 0.8 0
AML1a(μg) 0 0.2 0.4 0.8 1.6 0
d
Luciferase activity(fold)
12
10
8
6
4
2
0
AML1b + M-CSFR
AML1b + mut-M-CSFR
AML1a + mut-M-CSFR
M-CSFR


a
LTR
IRES
YFP
LTR
LTR
FLAG
AML1a
IRES
YFP
LTR
b
Percent survival
100
75
50
25
0
AML1a
YFP
P=0.0023
0
60
120
180
240
300
360
days after BMT
c
d
e
f
g
h
i
1cm
2
0
1cm
2
3
4
5


a
Marker
AML1a vector
21#SP
103#SP
105#SP
109#SP
110#SP
1,000bp
750bp
AML1a
b
Marker
103#SP
105#SP
108#SP
110#SP
21#SP
293T-AML1a
1,000bp
750bp
AML1a
c
500bp
250bp
β-actin
d
293T-AML1a
21#SP
110#SP
109#SP
105#SP
AML1a
β-actin

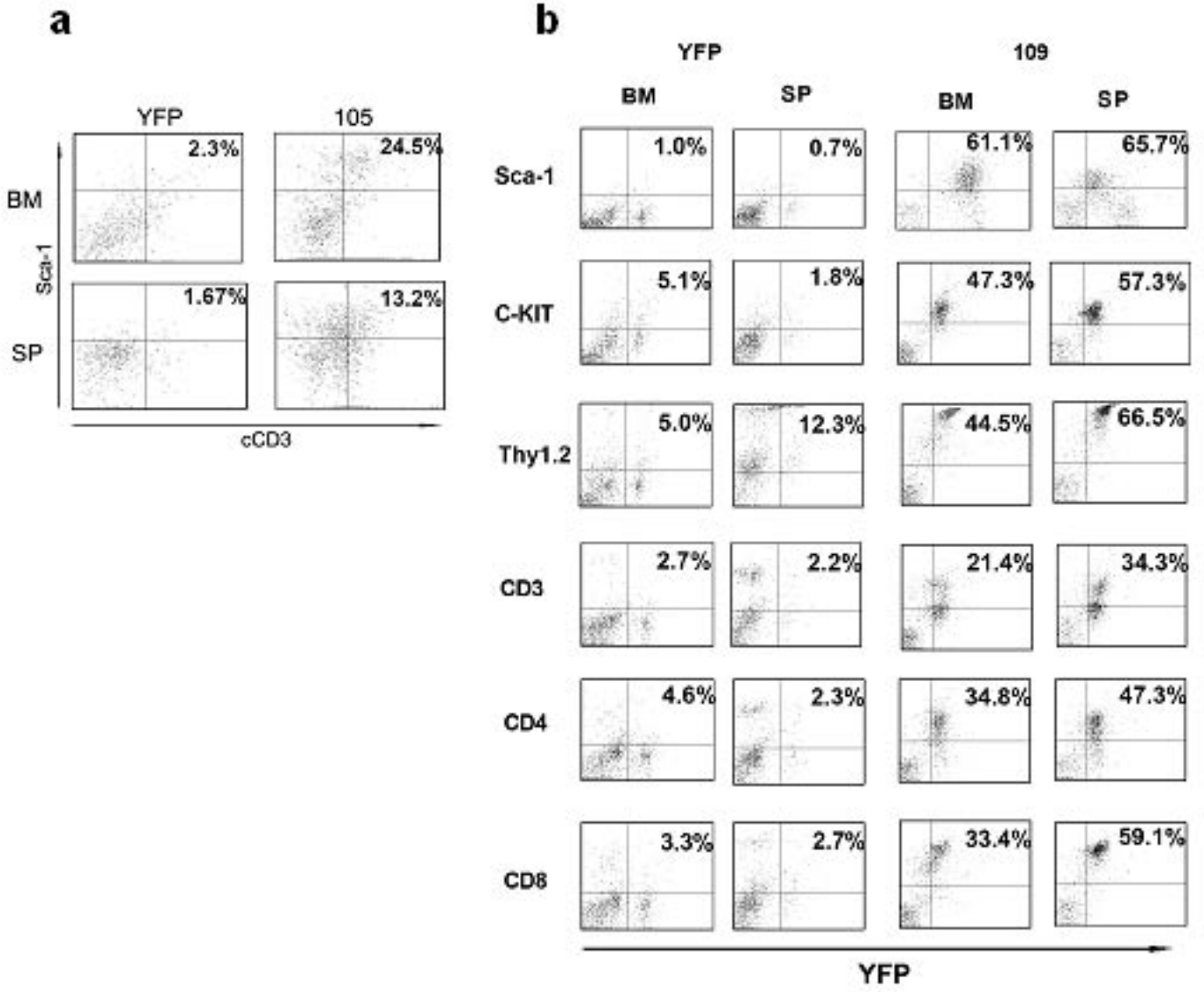

a
YFP
105
BM
SP
Sca-1
2.3%
24.5%
1.67%
13.2%
cCD3
b
YFP
109
BM
SP
BM
SP
Sca-1
1.0%
0.7%
61.1%
65.7%
C-KIT
5.1%
1.8%
47.3%
57.3%
Thy1.2
5.0%
12.3%
44.5%
66.5%
CD3
2.7%
2.2%
21.4%
34.3%
CD4
4.6%
2.3%
34.8%
47.3%
CD8
3.3%
2.7%
33.4%
59.1%
YFP

Table1: Clinical characteristics of 77 *de novo* AL patients.

Group	**AML1a+**	**AML1a-**
Gender		
Male	35	11
Female	25	6
Media age（years old）	25.5	26
FAB subtype		
ALL	15	6
AML	29	22
HAL	2	3
Media WBC（$\times 10^9$）		
ALL	30.58	21.93
AML	31.93	24.35
Media percentage of blast cells(%)		
ALL	88	73.5
AML	76.5	65.75
CD34 positive(case)		
ALL	12(13)	3(4)
AML	9(29)	5(13)
HLA-DR positive(case)		
ALL	11(14)	3(5)
AML	11(29)	9(14)

Table 2. Characteristics of *AML1a* induced leukemia mice

No. Mice	**101**	**102**	**103**	**105**	**108**	**110**	**107**	**109**	**111**
Survival time after BMT(d)	**110**	**133**	**142**	**180**	**180**	**234**	**282**	**282**	**312**
Phenotypes of BM and SP	**ND**	**ND**	**Sca-1 cCD3**	**Sca-1 cCD3**	**Sca-1 cCD3**	**Sca-1 c-Kit Thy1.2 CD3 CD4 CD8**	**Thy1.2 CD3 CD4 CD8**	**Sca-1 c-Kit Thy1.2 CD3 CD4 CD8**	**Sca-1 c-Kit Thy1.2 CD3 CD4 CD8**
Thymoma	**ND**	**ND**	**+**	**_**	**+**	**_**	**+**	**+**	**+**
SP weight(g)	**ND**	**ND**	**0.2**	**0.4**	**0.2**	**0.31**	**0.2**	**0.21**	**0.42**
WBC ($\times 10^6$/ml)	**10.1**	**12.7**	**15.2**	**12.7**	**12**	**15.2**	**22.6**	**23.4**	**30.2**
Diagnosis	**ALL**	**ALL**	**T-ALL**	**T-ALL**	**T-ALL**	**T-ALL**	**T -ALL**	**T-ALL**	**T-ALL**

ND: not determined