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Regulation of cancer stem cell properties by CD9 in human B-acute lymphoblastic leukemia

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ABSTRACT

Although the prognosis of acute lymphoblastic leukemia (ALL) has improved considerably in recent years, some of the cases still exhibit therapy-resistant. We have previously reported that CD9 was expressed heterogeneously in B-ALL cell lines and $CD9^+$ cells exhibited an asymmetric cell division with greater tumorigenic potential than $CD9^-$ cells. $CD9^+$ cells were also serially transplantable in immunodeficient mice, indicating that $CD9^+$ cell possess self-renewal capacity. In the current study, we performed more detailed analysis of CD9 function for the cancer stem cell (CSC) properties. In patient sample, CD9 was expressed in the most cases of B-ALL cells with significant correlation of CD34-expression. Gene expression analysis revealed that leukemogenic fusion proteins and Src family proteins were significantly regulated in the $CD9^+$ population. Moreover, $CD9^+$ cells exhibited drug-resistance, but proliferation of bulk cells was inhibited by anti-CD9 monoclonal antibody. Knockdown of CD9 remarkably reduced the leukemogenic potential. Furthermore, gene ablation of CD9 affected the expression and tyrosine-phosphorylation of Src family proteins and reduced the expression of histone-deubiquitinase USP22. Taken together, our results suggest that CD9 links to several signaling pathways and epigenetic modification for regulating the CSC properties of B-ALL.

1. Introduction

CD9 is a 24–27kD cell-surface glycoprotein which belongs to a tetraspanin super family, expressed in a variety of normal tissues, and reported to involve in cell adhesion, motility, and many signaling events [1]. In blood cells, although hematopoietic stem cells do not express CD9, pre-B lymphocytes express CD9 [2]. The importance of CD9 for cancer progression has often been indicated in recent years. CD9 was reported to be involved in invasion of cancer cells, and associated with cancer progression, metastasis, recurrence, and clinical outcome [3].

Although the prognosis of pediatric acute lymphoblastic leukemia (ALL) has improved considerably in recent years, a significant number of cases exhibit therapy-resistant. It has been postulated that the relapse may be due to cancer stem cell (CSC) [4]. CSC has self-renewal capacity and multipotency similar with normal stem cells, and proliferates by asymmetric cell division, generating mature leukemia cells. Therefore, CSC is believed to be a potential reason for chemoresistance, metastasis, and recurrence [5].

Previous studies using primary samples have shown that B-ALL cells capable of long-term proliferation *in vivo* were the $CD34^+/CD10^-/CD19^-$ fraction [6]. More recent study has also reported that CD133 was expressed on leukemia-initiating cells of childhood ALL [7]. This fraction was more resistant to anti-cancer drugs than the bulk leukemic cells.

To understand therapy-resistance in lymphoid leukemia, we have explored CSC markers using cell lines. In T-lineage malignancy [8], adult T-cell leukemia/lymphoma often contained SP cells [9]. We also found that CD90 and CD110 correlated with stem cell properties in T-ALL [10]. In B-ALL, we have previously reported that CD9 was expressed heterogeneously and only $CD9^+$ cells proliferated by asymmetric cell division-like manner *in vitro* [11]. $CD9^+$ cells also exhibited greater tumorigenic potential in immunodeficient mice than $CD9^-$ cells. Moreover, these $CD9^+$ cells were serially transplantable in mice and reconstituted the original pattern of CD9 expression, indicating that $CD9^+$ cell possesses the self-renewal potential.

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In this report, we conducted more detailed analysis of the CSC properties in CD9-expressing cells of B-ALL cell lines and patient samples. We found that leukemia-related genes and Src family genes were markedly regulated between $CD9^+$ and $CD9^-$ populations. We also found that CD9 is a promising target for CSC-oriented therapy by monoclonal antibody treatment and RNAi-knockdown. Moreover, ablation of CD9 significantly affected the expression and tyrosine-phosphorylation of Src family and histone ubiquitination through novel CSC marker ubiquitin-specific protease 22 (USP22) [12].

2. Materials and methods

2.1. Cell lines, culture, flow cytometry, and cell sorting

The protocols were described in our previous paper [11].

2.2. Patient specimens and antibodies

Diagnostic bone marrow or peripheral blood samples were obtained from pediatric patients with precursor B-ALL as described in Supplementary Table 1 (Approval number: 20-32-1006). For phenotypic analysis and transplantation, mononuclear cells were first gated by PI and lineage-markers mixture (APC-conjugated anti-CD3, CD33, CD56, and Immunoglobulin light chain κ/λ), then analyzed using anti-CD9-PE and CD34-FITC antibodies. For RT-PCR analysis, the lineage-negative cells were stained with CD9 and separated to extract RNA. Antibodies are listed in Supplementary Table 2.

2.3. Transplantation assay

All *in vivo* studies were approved by the Institute and Animal Care and Use Committee of the University of Tokyo. The protocol was described in our previous paper [11].

2.4. RT-PCR

Total RNA was extracted using RNeasy-Mini-Kit (Qiagen). RT reaction was conducted with Superscript III system (Invitrogen). PCR was conducted with the primers as described in Supplementary Table 2. PCR was performed with KOD-plus-DNA polymerase (Toyobo, Osaka). Thermal cycling was performed in 98 °C for 10 s and 68 °C for 30 s.

2.5. Oligonucleotide microarray analysis

Microarray analysis of the cell lines was performed according to the standard protocol using Human Genome U133A plus 2.0 Arrays (Affymetrix). Data mining was conducted using Gene Spring system (Agilent Technologies, Palo Alto). Analysis of the patient samples was done using 3D-Gene Human Oligo chip 25 k (TORAY, Tokyo).

2.6. shRNA lentiviral transduction

CD9-targeted shRNA lentiviral plasmid (MISSION; Sigma–Aldrich, target sequence: ccgggctgttcggatttaacttcatctcgagatgaagttaaatccgaacagctttttg) and non-targeting control plasmid (pLKO.1-puro) were cotransfected with ViraPower Lentiviral packaging mix to 293FT cells using Lipofectamine2000 (Invitrogen). The cells were transduced with the shRNA-expressing lentivirus, and stable cell lines were generated by selection with puromycin.

2.7. Cell cycle analysis

Cells (5×10^5) were first stained with CD9-FITC antibody and fixed in 70% ethanol. The cells were subsequently washed and added with RNase (final 10 μg/ml) and PI (10 μg/ml), then they were analyzed by FACS. The proportion of G2/M phase was calculated by the Watson pragmatic model with FlowJo software.

2.8. Drug-sensitivity assay

Cells (2×10^4 cells/ml) were seeded for 24 h in 96-well plates at 100 μl/well, then a total of 12 chemotherapeutic drugs (dexamethasone, cyclophosphamide, cisplatin, mitomycin C, daunorubicin, doxorubicin, methotrexate, Ara-c, 5-FU, etoposide, 5-azacitidine, and vincristine) were employed to test the drug-sensitivity and their 50% inhibitory concentration. After 72 h, cell viability was checked by CellTiter96 kit (Promega). Additionally, cells were cultured in 25 cm^2 flask and the sensitive drugs were added to their respective 50–70% inhibitory concentration (25 nM for dexamethasone, 2.0 μM for cisplatin, and 5.0nM for vincristine), and incubated for 72 h to analyze CD9-expression.

2.9. Growth inhibition by monoclonal antibody

YAMN90 cells (2×10^3 cells/well in 200 μl) were seeded into the 96-well plates. Anti-CD9 (5H9) antibody was then added to make final concentration of 1.0 and 10.0 μg/ml ($n = 6$). After 48 h, MTT assay was performed using TetraColorONE system (Seikagaku Bioscience, Tokyo).

2.10. Western blot analysis

Cells were collected and suspended in lysis buffer (20 mM Tris–HCl pH7.5, 150 mM NaCl, 1%NP40, 1 mM Na_3VO_4, 1 mM NaF, protease inhibitors). Detection of proteins was carried out using ECL advance system (GE, UK).

3. Results

3.1. Correlation between CD9 and CD34-expressions in pediatric B-ALL clinical samples

In our previous report, we used three B-ALL cell lines to analyze stem cell properties of B-ALL both *in vitro* and *in vivo* assays [11]. We conducted further analysis using several primary samples of childhood precursor B-ALL patients to examine the expression of CD9 and CD34. We obtained eight primary samples (peripheral blood and bone marrow), whose characteristics are summarized in Supplementary Table 1. As shown in Fig. 1, both CD9 and CD34 were heterogeneously expressed in all cases. Moreover, most $CD34^+$ cells also co-expressed CD9 in patients 2, 4, 5, and 7 (Fig. 1A, rectangles). As previous studies have reported that CSCs of primary B-ALL cells were contained in the $CD34^+$ populations [6], our data suggest that CSCs of patient samples are also significantly enriched in the $CD9^+$ fraction.

3.2. Transplantation assays of primary samples

We next conducted transplantation assay of primary B-ALL samples into immunodeficient NOG mice. Five patient samples (patients 3, 4, 6, 7, 8) were selected for evaluation of engraftment potential. In each experiment, separated $CD9^+$ and $CD9^-$ cells (1×10^5 cells each) were transplanted into three mice, and the animals were then monitored over the indicated time periods. The mice were subsequently sacrificed for an analysis of the status of

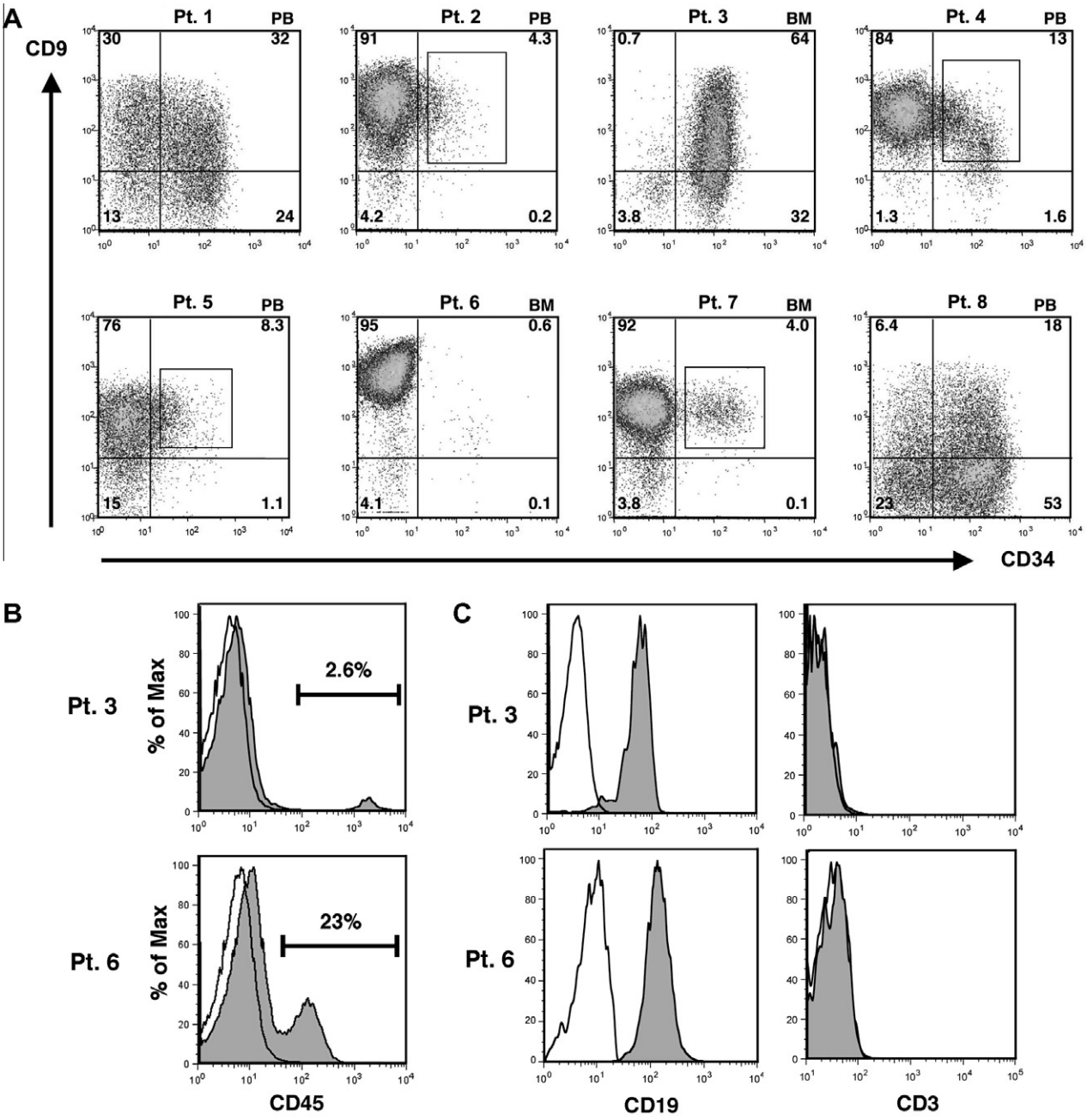

Fig. 1. (A) Analysis of primary samples. Significant correlation between CD9 and CD34-expressions were observed in four patient samples (rectangles). Numbers in the corner depict the percentage of cell number in each fraction. PB, peripheral blood. BM, bone marrow. (B) Transplantation experiments of patient samples. The peaks representing $CD9^+$ engraftments are shown in gray, and $CD9^-$ engraftments are represented as translucent peaks on the overlay histograms. (C) Expressions of CD3 and CD19 in the $CD45^+$ cells.

human-derived cells in the bone marrows by staining with anti-human CD45 antibody at three time points (3rd, 5th, and 7th weeks), with the results obtained as follows.

There was no detectable human cell in engrafted mice from samples of patients 4, 7, and 8, at any time points. For samples from patients 3 and 6, while obvious engraftment was not observed until the 5th week, we did detect clear differences between $CD9^+$ and $CD9^-$ cell engraftments in the bone marrow at the 7th week time point. In both cases, although there was no human cell in the mice with $CD9^-$ engraftment, the mice with $CD9^+$ engraftment contained human cells (2.6% and 23% of $CD45^+$ cells from patients 3 and 6, respectively) (Fig. 1B).

We also stained the $CD45^+$ population with anti-human CD3 and CD19, and confirmed that the $CD45^+$ cells were truly of B-lineage cells (CD19-positive) but not T-lineage cells (CD3-negative) (Fig. 1C). These results suggest that $CD9^+$ cells of the primary samples possess higher CSC potentials than $CD9^-$ cells.

3.3. Gene expression analysis between $CD9^+$ and $CD9^-$ cells

To distinguish differences between $CD9^+$ and $CD9^-$ cells in terms of the molecular pathways involved in CSC potentials, we evaluate differential gene expression profiles by performing DNA microarray analysis of the cell lines. Using Affymetrix Gene Chip, we found that many genes were up or down-regulated. Among the regulated genes, we found that several genes related to leukemia and cancer signal were differentially regulated between $CD9^+$ and $CD9^-$ cells. Among the genes up-regulated in $CD9^+$ cells of YAMN90 (*t*(1;19)-po-

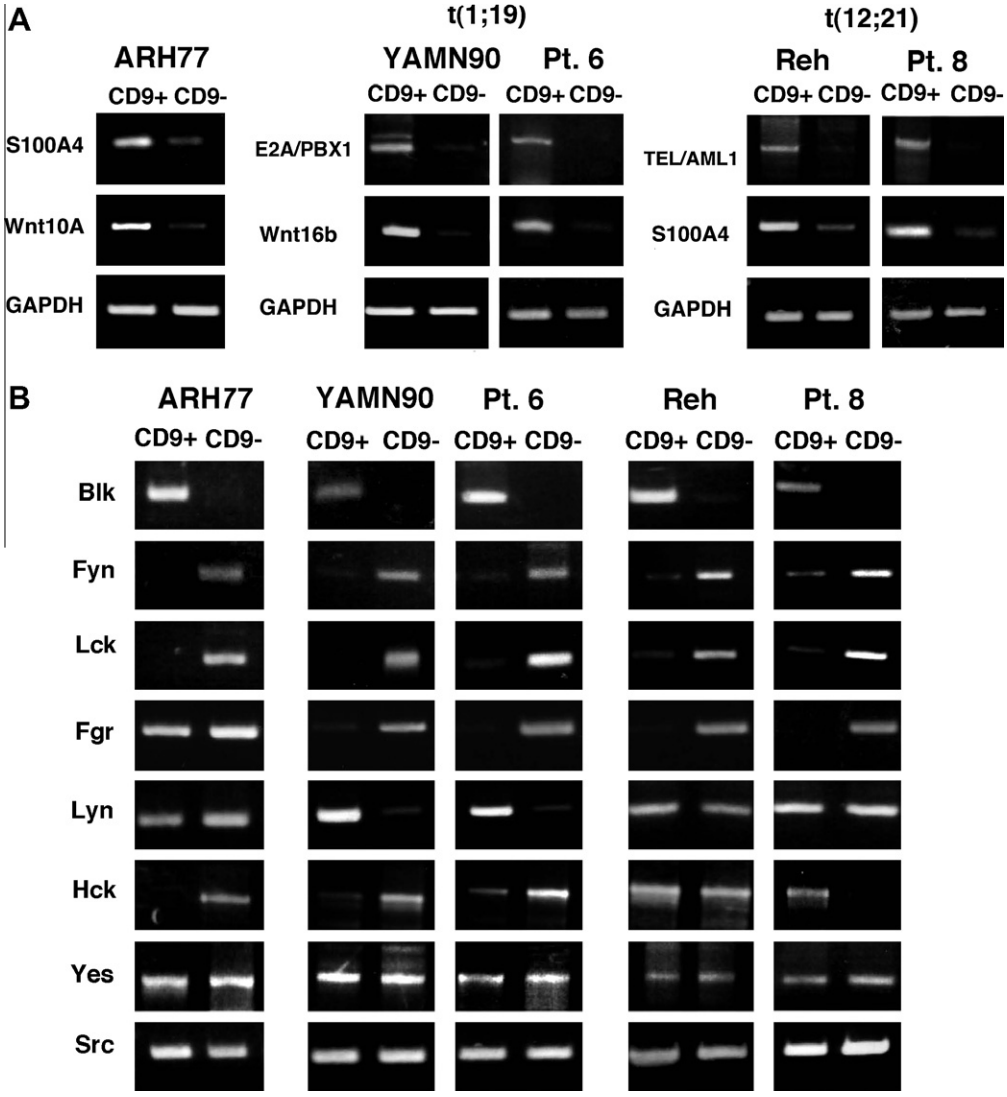

Fig. 2. RT-PCR analysis of the CD9-isolated cells. (A) Several genes related to cancer and chromosomal translocation were differentially expressed between $CD9^+$ and $CD9^-$ cells. (B) Expression of Src family proteins. Several Src family proteins were significantly regulated.

sitive), we found that the chromosomal translocation gene product of E2A/PBX1 were significantly up-regulated (fold change, 3.8×). In ARH77 and Reh, S100A4 (S-calcium binding protein A4) [13], associated with migration and metastasis, was commonly up-regulated (5.8× in ARH77 and 2.3× in Reh). In addition, a cancer-related gene Wnt10A [14] was also up-regulated in ARH77 (3.0×).

The microarray data of each cell line was further confirmed by RT-PCR, with the same results obtained for these genes (Fig. 2A). We then analyzed the expression of another member of Wnt family protein, Wnt16b, which has been reported to be overexpressed in *t*(1;19)-positive leukemia [15]. RT-PCR analysis confirmed that Wnt16b was highly expressed in $CD9^+$ cells of YAMN90. The chromosomal translocation gene product of TEL/AML1 was also highly expressed in $CD9^+$ cells of Reh (*t*(12;21)-positive).

We further analyzed the expression of genes up-regulated in YAMN90 and Reh using patient samples containing the same karyotype as the corresponding cell lines (Fig. 2A). Samples of patients 6 and 8 were selected for RNA extraction, since they possess the *t*(1;19, E2A/PBX1) and *t*(12;21, TEL/AML-1) translocations, respectively. We were also able to confirm the up-regulation of these genes in $CD9^+$ cells of these primary samples. These results suggest that genes up-regulated in $CD9^+$ cells, especially products of chromosomal translocation, are important for CSC potentials of B-ALL.

3.4. Expressions of Src family proteins

We next performed the same analysis of sample from patient 6 using a 3D-Gene Human-Oligo-Chip. Among the genes regulated, we found that four members of Src family protein exhibited significant regulation in the $CD9^+$ fraction (Blk, 7.5×; Fyn, 0.19×; Lck, 0.15×; Fgr, 0.22×). Since Src family proteins play key roles in signal transduction of B-lymphocyte and tumorigenesis of various malignancies [16], we further performed RT-PCR analysis to confirm the microarray data.

As shown in Fig. 2B, Blk was up-regulated, but Fyn and Lck were down-regulated in $CD9^+$ cells of all samples, consistent with the microarray data. Fgr was down-regulated except in ARH77. We also examined other Src family proteins and found that Lyn was up-regulated only in the *t*(1;19)-positive samples. However, Yes and Src were equally expressed in each sample set. These results hence suggest that regulation of various molecular pathways by Src family proteins plays an important role in CSC potentials.

3.5. Drug-sensitivity assay

As drug-resistance is one of the important characters of CSC, we next examined the relationship between CD9-expression and

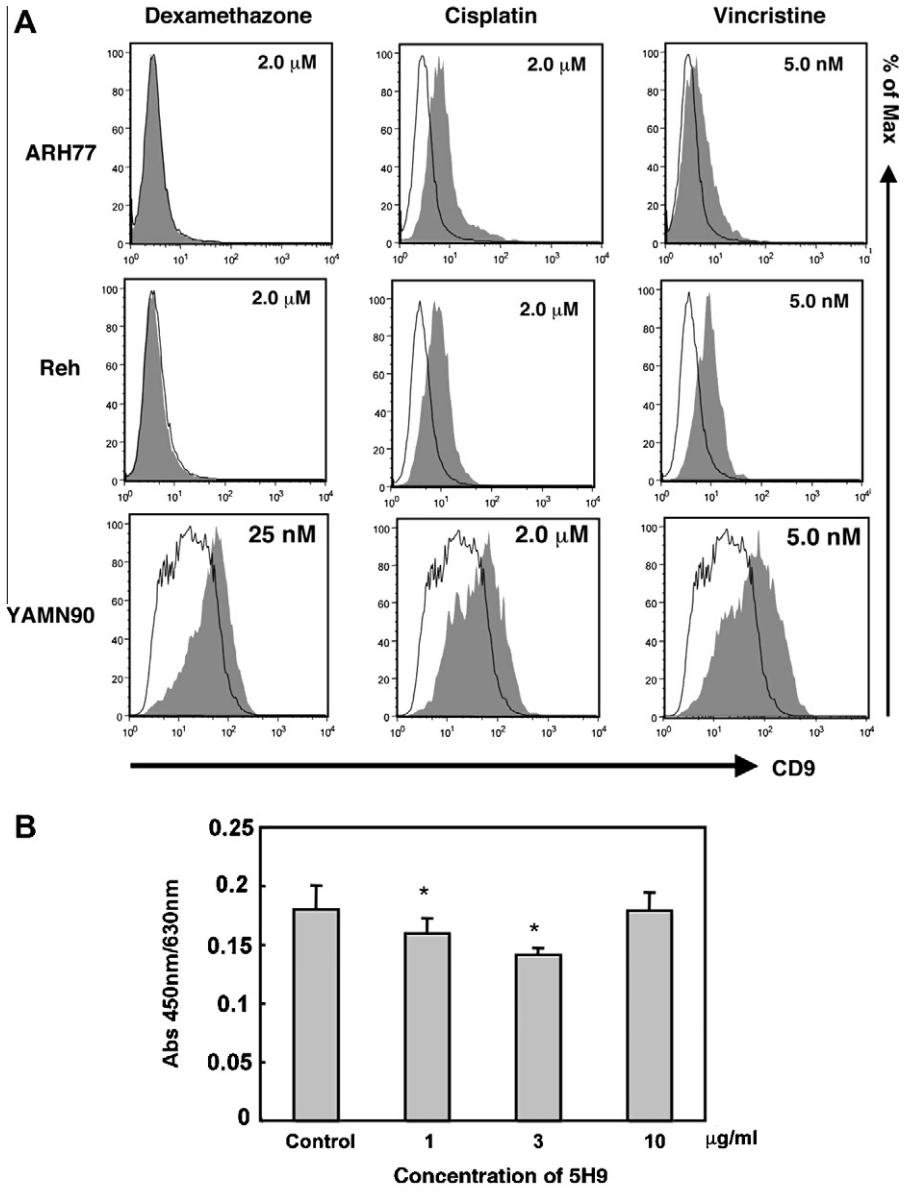

Fig. 3. (A) Effect of anti-cancer drugs for CD9-expression. (B) Growth inhibition by anti-CD9 antibody in YAMN90 cells. Proliferation was significantly inhibited at the concentration of 1.0 and 3.0 μg/ml. *Statistically significant.

drug-resistance. Standard chemotherapy for remission induction against pediatric B-ALL is the combination of dexamethasone, vincristine, and L-asparaginase [17]. We first examined drug sensitivity in these cell lines for 12 anti-cancer drugs (see Section 2). After the 3 days of incubation, the proportions of living cell were analyzed. We found that these cell lines actually showed drug-resistance against several drugs such as cisplatin, vincristine, and dexamethasone. Moreover, incubation with cisplatin (2.0 μM) and vincristine (5.0 nM) significantly increased the proportion of $CD9^+$ cells (Fig. 3A). This means that mainly $CD9^-$ cells died but $CD9^+$ cells survived. In the case of dexamethasone, ARH77 and Reh cells exhibited complete drug-resistance for dexamethasone (>2.0 μM), but the proportion of $CD9^+$ cells in YAMN90 dramatically increased (<25 nM). These results indicate that $CD9^+$ cells are relatively more drug-resistant than $CD9^-$ cells.

3.6. Growth inhibition by anti-CD9 monoclonal antibody

Targeting cell surface CSC markers by monoclonal antibodies is a promising strategy for future CSC-oriented therapy. We have already developed the monoclonal anti-human CD9 antibody (5H9) and confirmed anti-proliferative effect on T-cells [18]. Therefore, we next examined the effect of 5H9 against YAMN90 cells, which express CD9 at a significant high level.

YAMN90 cells were incubated with 5H9 at the concentrations between 1.0 and 10 μg/ml (Fig. 3B). After 2 days, MTT-assay was performed to estimate the cell proliferation. Although higher concentration of the antibody (10 μg/ml) exhibited no effect for proliferation due to antibody excess phenomenon, we found that proliferation was significantly inhibited *in vitro* by dose-dependent manner at 1.0 and 3.0 μg/ml. The maximum inhibition was 22% at 3 μg/ml of 5H9. This result suggests that anti-CD9 monoclonal antibody can be effective for clinical application if leukemia cells express CD9. Moreover, such antibodies may be able to eradicate CSCs for consequent complete remission.

3.7. Effect of CD9-knockdown for cell proliferation and survival of mice

To assess the functional significance of CD9 in B-ALL cells, we targeted CD9 expression by small interference RNA (siRNA) using

lentivirus expressing short hairpin RNA (shRNA). Treatment of CD9-shRNA caused more than 90% reduction of $CD9^+$ population relative to control-shRNA in these cell lines (Fig. 4A). Once knockdown of CD9 was confirmed in the cells, this effect continued at lease 1 month of culture even in the absence of puromycin.

Next, we performed cell cycle analysis to compare proliferation potential of CD9-isolated cells. As shown in Fig. 4B, $CD9^+$ cells contained G2/M-phase cells in significantly higher levels than $CD9^-$ in all cell lines. We also examined the siCD9 cells of YAMN90 and found that G2/M-phase cells were also reduced significantly. As G2/M-phase represents the proliferating phase, knockdown of CD9 reduced the proliferation potential significantly.

Using the CD9-knockdown cells of YAMN90, transplantation assay was performed to evaluate the leukemogenic potential and effect on survival (Fig. 4C). The siCD9 and control cells (1×10^6 cells) were transplanted into the mice, and the animals were then observed until leukemic death or 100 days. The Kaplan–Meier survival curves demonstrated a clear difference between the two groups ($p = 0.027$). Mice injected with the control cells died from leukemia within 41–67 days, while only two mice injected with the siCD9 cells died within 100 days. Pathological analysis revealed that the mice with control cells had marked splenomegaly (average weight, 0.106 g) and bone marrow involvement, but the mice with siCD9 cells had no splenomegaly (average weight, 0.011 g). These results indicate that knockdown of CD9 reduces the leukemogenic potential and suggest that CD9 plays an important role in the progression of B-ALL *in vivo*.

3.8. Regulation of Src family proteins in CD9-knockdown cells

As knockdown of CD9 clearly demonstrated that CD9 was involved in the leukemogenic potential, we examined the effect of CD9-knockdown on cellular signaling pathways. We used YAMN90, because other cell lines contained only a small proportion of CD9-expressing cells (2.8–4.4%) but majority of YAMN90 cells (76.7%) express CD9 (Fig. 4A). RT-PCR analysis of Src family proteins in

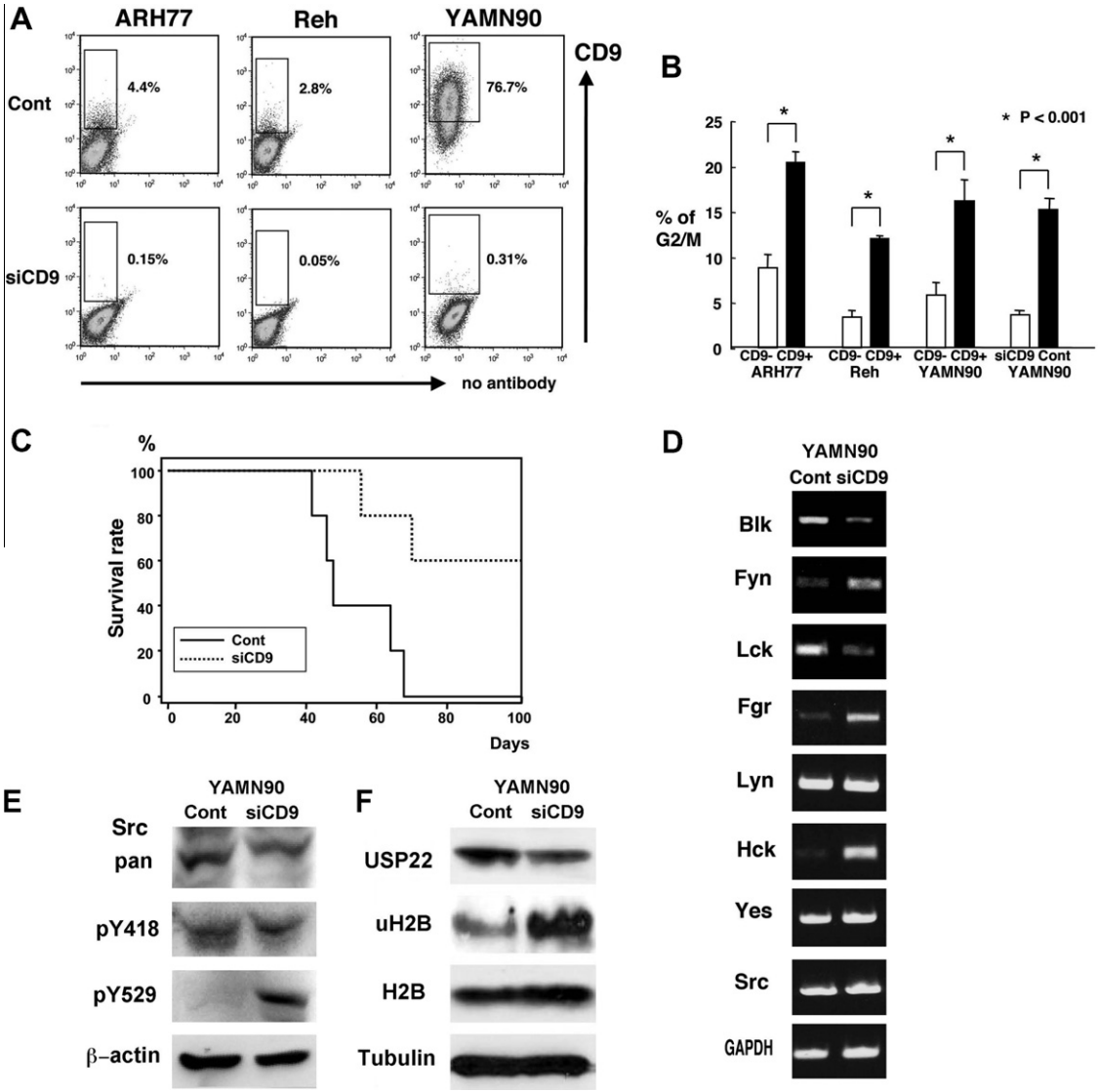

Fig. 4. (A) Knockdown of CD9-expression by shRNA. The expression of CD9 was significantly reduced by CD9-shRNA (siCD9) compared to the control-shRNA (Cont). (B) Cell cycle analysis. $CD9^+$ cells contained significantly higher G2/M proportion than $CD9^-$ cells. Similar result was obtained in CD9-shRNA cells of YAMN90. (C) Transplantation assay of the shRNA cells of YAMN90. CD9-knockdown clearly induced longer survival of mice. (D) Expression of Src family proteins in the CD9-knockdown cells. The shRNA cells exhibited quite similar patterns with the $CD9^+$ and $CD9^-$ cells of YAMN90. (E) Tyrosine-phosphorylation of Src protein. Phosphorylation of Src-Y418 exhibited no significant difference, whereas phosphorylation of Y529 was significantly up-regulated in the CD9-knockdown cells. (F) Epigenetic effect of CD9-knockdown. USP22 was down-regulated in the CD9-knockdown cells. Consequently, uH2B was markedly up-regulated.

CD9-knockdown (Fig. 4D) revealed that their expression patterns were quite similar with those of $CD9^+$ and $CD9^-$ cells (Fig. 2B).

Next, tyrosine-phosphorylation of Src family proteins in the CD9-knockdown cells was examined by western blotting (Fig. 4E). We focused on Src protein because its mRNA expression was not affected in the CD9-knockdown cells (Fig. 4D). In Src protein, tyrosine 418 (Y418) and Y529 are known to be critical phosphorylation sites [19]. Full catalytic activity requires phosphorylation of Y418, but phosphorylated Y529 acts as the negative regulator of Src function. Although phosphorylation of Y418 showed no obvious difference between the CD9-knockdown and control cells, Y529 was significantly phosphorylated in the CD9-knockdown cells, indicating that Src protein was inactivated. Taken together, these results suggest that the expression of CD9 links to the expression and phosphorylation of Src family proteins.

3.9. Epigenetic effect of CD9-knockdown cells

Finally, we examined the epigenetic effect of CD9-knockdown. USP22 is known to be a deubiquitinase of histone H2B and a subunit of SAGA complex, a multiprotein transcriptional coactivators complex [12]. This molecule was previously identified as one of the 11-gene signatures that consistently display a stem cell-resembling expression profile in distant metastatic lesions, suggesting that USP22 is a putative CSC marker [20].

Using the shRNA cells of YAMN90, the expression of USP22 was compared by western blotting. As shown in Fig. 4F, USP22 was significantly decreased in the CD9-knockdown cells. Next, ubiquitination of H2B was examined for further confirmation of this effect, because USP22 is a deubiquitinase for ubiquitinated H2B (uH2B). We found that uH2B was dramatically up-regulated in the CD9-knockdown cells, supporting the notion that CD9 links to the epigenetic modification in CSC through USP22.

4. Discussion

In our previous report, $CD9^+$ cells were suggested to be the stem cell-like subpopulation of B-ALL having the leukemogenic and self-renewal potentials [11]. In this report, we showed that CD9 was expressed with highly correlation of CD34 and correlated with the engraftment potential in pediatric patient samples. Regulation of cancer-related genes was observed in the $CD9^+$ cells, and the $CD9^+$ cells exhibited the drug-resistance. Moreover, CD9-knockdown reduced the leukemogenic potential in the transplanted mice. Taken together, our present study further demonstrated that CD9 plays important roles in the CSC properties of B-ALL and can be a potential clinical parameter.

On the other hand, *t*(1;19, E2A/PBX1) and *t*(12;21, TEL/AML1), commonly found chromosomal translocations in pediatric B-ALL, were observed in the cell lines and corresponding primary samples. Our data demonstrated that mRNA of TEL/AML1 and E2A/PBX1 products were significantly up-regulated in the $CD9^+$ populations of both cell lines and primary samples sharing the same respective translocations [15]. While the exact role played by specific chromosomal translocation products in the regulation of CSC potentials has not yet been elucidated, it is known that cord blood $CD34^+$ cells expressing AML1/ETO can proliferate in the absence of stroma for over 7 months [21]. This result suggests that a chromosomal translocation product can affect the microenvironment-dependent proliferation of CSCs.

Another intriguing finding is that CD9 is associated with the epigenetic modification for CSC signatures through the putative CSC marker USP22. Knockdown of CD9 correlated with lower expression of USP22, suggesting that USP22 requires CD9-expression. Consistent with USP22 as a deubiquitinase of uH2B, uH2B-levels were dramatically increased in the CD9-knockdown cells, indicating that CD9 regulates epigenetic modification for CSC functions. Importantly, this is the first demonstration that cell surface molecule CD9 appeared to be linked with the nuclear transcriptional component, for regulating the epigenetic modification in CSC properties.

Moreover, significant regulations in the gene expression and tyrosine-phosphorylation of Src family proteins were observed in the $CD9^+$ and CD9-knockdown cells. Src family proteins are tyrosine kinases and have many biological functions in not only B-cell but also B-ALL cells [22]. In the case of Src protein, the expression was not affected by CD9-knockdown, but Y529 was significantly phosphorylated.

There are two critical tyrosine-phosphorylation sites of Src, Y418 and Y529 [19]. Although Y418 is one of the autophosphorylation sites and full catalytic activity requires its phosphorylation, Y529 acts as the negative regulator. When Y529 is phosphorylated, Src is held in the inactive form by blocking phosphorylation of Y418. As phosphorylated Y529 acts as the negative regulator, knockdown of CD9 eventually prevents Src activation. Therefore, our findings suggest that CD9 tightly links to the regulation of Src family proteins, and also indicating that Src family proteins play an important role in the CSC potentials. Moreover, if certain Src family inhibitors that can selectively reduce $CD9^+$ population are discovered, such molecule may be able to eradicate leukemia cells.

Another therapeutic procedure against CSC is to target cell surface molecules specifically expressed on CSC by monoclonal antibodies. In the case of human AML, several specific CSC markers are highly expressed on proposed CSC, and application of their monoclonal antibodies could inhibit the progression of leukemia in mouse engraft models [23]. Currently, several monoclonal antibodies against certain leukemia-associated antigens have also been already incorporated into various anti-leukemic regimens [23].

Until now, we have already developed anti-CD9 monoclonal antibody, 5H9. In our previous study, 5H9 has been shown to inhibit the proliferation of stimulated T-cells and induce apoptosis of Jurkat T-cells [18]. Our present study also showed that 5H9 has significant inhibitory effect on the proliferation of B-ALL cells. Therefore, targeting for CD9 by monoclonal antibody seems to be promising strategy against many cases of ALL.

In conclusion, expression of CD9 is well-correlated with the CSC potentials and involved in the progression of B-ALL. Moreover, Src family proteins and USP22 are implicated to be intimately involved in the CSC signals. Therefore, targeted therapies against CD9 and its downstream signals will be novel therapeutic approaches in the near future.

Acknowledgments

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.04.098.

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