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Differential expression of the novel oncogene, *SALL4*, in lymphoma, plasma cell myeloma, and acute lymphoblastic leukemia

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SALL4, a newly identified zinc-finger transcriptional factor important for embryonic development, is mapped to chromosome 20q13. Previously, we reported that *SALL4* was constitutively expressed in acute myeloid leukemia and *SALL4* transgenic mice developed acute myeloid leukemia. In this study, we aimed to survey *SALL4* protein expression in benign and neoplastic hematopoietic tissues in addition to acute myeloid leukemia using immunostaining with a polyclonal anti-*SALL4* antibody. Primary hematological tumors (178) and 15 benign hematopoietic tissues were examined. Reverse transcription-polymerase chain reaction was also performed to detect *SALL4* mRNA expression on eight precursor B-cell lymphoblastic leukemia/lymphomas, 10 benign hematopoietic tissues, and seven hematopoietic cancer cell lines. Of the benign tissues, *SALL4* expression was detectable only in CD34$^+$ hematopoietic stem/progenitor cells (2/2 at protein level, 3/3 at RNA level). In neoplastic tissues, only precursor B-cell lymphoblastic leukemia/lymphomas had detectable *SALL4* (12/16 at protein level, 7/8 at RNA level), similar to that observed in acute myeloid leukemia. Of the seven cell lines examined, only those derived from acute myeloid leukemia and precursor B-cell lymphoblastic leukemia/ lymphomas were positive. To conclude, *SALL4* expression is normally restricted to CD34$^+$ hematopoietic stem/ progenitor cells. The persistence of *SALL4* expression in leukemic blasts in precursor B-cell lymphoblastic leukemia/lymphomas resembles to what we observed in acute myeloid leukemia, and correlates with the maturation arrest of these cells. We have shown in our previous study that the constitutive expression of *SALL4* in mice can lead to acute myeloid leukemia development. The similar expression pattern of *SALL4* in acute myeloid leukemia and B-cell lymphoblastic leukemia/lymphomas suggests that these two disease entities may share similar biological features and/or mechanisms of leukemogenesis. More definite studies to investigate the role of *SALL4* in the pathogenesis of B-cell lymphoblastic leukemia/lymphomas are needed in the future to address this question.
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SALL1 through *SALL4* in the *SALL* gene family are human homologs of *Drosophila spalt* (*sal*).[1–4] In *Drosophila*, *spalt* is a homeotic gene that is essential for the development of posterior head and anterior tail segments. It plays an important role in terminal differentiation of photoreceptors, tracheal development,[5] and wing vein placement.[6] *Sal*-related genes have been isolated from *Caenorhabditis elegans*,[7] fish,[8,9] Xenopus,[10,11] mice,[12,13] and humans.[14] Each of these homologs is expressed during embryonic development and in specific adult tissues.

In humans, *SALL1* is mutated in patients with Townes-Brocks syndrome, with phenotypes involving urogenital, limb, anal, and cardiac malforma-

tions.[15–19] Human *SALL4* mutations are associated with Duane radial ray syndrome (or Okihiro syndrome).[3,20–22] Duane radial ray syndrome is a human autosomal-dominant syndrome involving radial-sided hand anomalies in association with Duane syndrome. Duane syndrome is characterized by an eye movement disorder (strabismus) in which eye abduction, or adduction, or both, is limited. Defects in hematopoiesis have not been reported to date in patients with Townes-Brocks syndrome or Duane radial ray syndrome. *SALL3* is mapped to chromosome 18q23, and may be involved in the phenotype of patients with 18q deletion syndrome, which is characterized by developmental delay, midface hypoplasia, hearing loss, and tapered fingers.[23] Unlike the other *SALL* gene family members, no known congenital malformations have been associated with *SALL2* mutation.[24] We have shown, however, that *SALL2* is involved in normal hematopoiesis, while others have suggested that it is involved in tumorigenesis.[25–28]

We recently found that *SALL4* expression is constitutively expressed in all cases of acute myeloid leukemia.[29] To further understand the biological importance of this novel oncogene in other hematopoietic malignancies, we performed an analysis of *SALL4* expression in normal and neoplastic hematopoietic tissues using both reverse transcription-polymerase chain reaction and immunostaining. We found a preferential expression of *SALL4* in normal purified CD34$^+$ hematopoietic stem/progenitor cells, leukemic blasts in precursor B-cell lymphoblastic leukemia/lymphomas, as well as acute myeloid leukemia. The biological importance of these findings is discussed.

Materials and methods

Tissue and Tumor Samples

Normal and neoplastic lymphoid tissue samples were randomly retrieved from the files of the Department of Laboratory Medicine and Pathology at the Cross Cancer Institute, the University Alberta, and the University of Arkansas. Two cases of normal CD34$^+$ hematopoietic stem/progenitor cells were selected from mobilized peripheral blood of normal donors whose recipients died before the use of these cells, thus were considered discarded tissues. The other three cases were purchased from Cambrex Bio Science Walkersville Inc. (Walkersville, MD, USA). The use of these tissues has been approved by the Institutional Review Board. Normal tissue samples included benign reactive lymph nodes ($N=2$), thymus ($N=2$), spleen ($N=2$), tonsils ($N=4$), bone marrow aspirates ($N=2$), bone marrow trephine biopsy ($N=3$), and peripheral blood mononuclear cells ($N=5$). Neoplastic tissue samples were derived from a total of 186 patients, and they included (1) malignant lymphomas ($N=113$ in total): non-Hodgkin's lymphoma ($N=108$) with 42 T-cell and 66 B-cell; and Hodgkin's lymphoma ($N=5$) with four classical and one lymphocyte predominant type; (2) lymphoblastic leukemia/lymphomas ($N=36$), with 24 precursor B-cell and 12 precursor T-cell type; (3) plasma cell myelomas ($N=32$); and (4) acute myeloid leukemias ($N=5$), which served as positive controls. Within the group of B-cell lymphomas, there were follicular lymphomas ($N=7$), small lymphocytic lymphomas ($N=30$), marginal zone lymphomas ($N=9$), mantle cell lymphomas ($N=5$), diffuse large B-cell lymphomas ($N=10$), and Burkitt's lymphomas ($N=5$). Within the group of T-cell lymphomas, there were ALK-anaplastic large-cell lymphoma ($N=39$), peripheral T-cell lymphoma ($N=2$), and extranodal T/NK-cell lymphoma ($N=1$). Diagnoses for all cases were based on morphological and immunophenotypic criteria according to the WHO classification system of hematopoietic neoplasms.

With a few exceptions, all of the tissue samples were formalin-fixed and paraffin-embedded, and they were subjected to immuohistochemical studies for *SALL4* (described below). Exceptions were eight cases of precursor B-cell lymphoblastic leukemia/lymphomas, all of which had >70% blasts in the aspirates, two of the five bone marrow aspirates, all five samples of peripheral blood mononuclear cells, and all five samples of HSC/HPC, all of which were fresh tissue samples that were preserved in 10% dimethyl sulfoxide. All of the fresh samples of precursor B-cell lymphoblastic leukemia/lymphomas, bone marrow aspirates, peripheral blood mononuclear cells, as well as three of five cases of CD34$^+$ hematopoietic stem/progenitor cells were subjected to reverse transcription-polymerase chain reaction (method described below). The remaining two CD34$^+$ hematopoietic stem/progenitor cells samples were subjected to immunocytochemical staining. Tissue microarrays incorporating triplicate cores of all cases of anaplastic large cell lymphoma, plasma cell myeloma, acute myeloid leukemia, and small lymphocytic lymphoma were used for immunohistochemistry. A manual tissue arrayer (Beecher Instruments, WI, USA) was used to construct the tissue arrays. Whole tissues were used for the remaining paraffin-embedded cases.

Cell Lines and Cell Culture

Each of the following seven ATCC cell lines was subjected to reverse transcription-polymerase chain reaction to detect *SALL4* expression. A total of 1×10^6 cells were used for extraction of mRNA: RL and BJAB, derived from B-cell lymphoma; SUP-M2, derived from ALK$^+$ anaplastic large T-cell lymphoma; Reh, derived from precursor B-cell lymphoblastic leukemia/lymphomas; JURKAT, derived from T-cell lymphoblastic leukemia/lymphomas; MMIS, derived from plasma cell myeloma; and Kasumi-1, derived from acute myeloid leukemia. All of these

cell lines were stored in liquid nitrogen and cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 2 mmol/l glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin.

Magnetic Nanoparticles for Selecting Normal CD34$^+$ Hematopoietic Stem/Progenitor Cells and Flow Cytometry for Assessing Purity and Viability

To examine the expression of *SALL4* gene and protein in normal CD34$^+$ hematopoietic stem/progenitor cells, magnetic nanoparticles (StemCell Technologies, Vancouver, BC, Canada) was used to positively select CD34$^+$ cells from previously frozen normal mobilized peripheral blood.

Frozen cells were thawed at 37°C in the water bath. Mononuclear cells were prepared using Ficoll density separation (StemCell Technologies), and then washed for 5 min in PBS containing 2% FBS and 1 mM EDTA (StemCell Technologies). Clumpy suspension was filtered through a 70 μm mesh nylon strainer (BD Biosciences, San Diego, CA, USA). Mononuclear cell suspensions at a concentration of 1×10^8 cells/ml were placed in 12×75 mm polystyrene tube to properly fit into the Magnet rack. CD34 antibody (clone QBend 10) was then added, and magnetic selection was completed according to the manufacturer's instructions (StemCell Technologies).

Purity of the positively selected cells was assessed using phycoerythrin (PE)-conjugated CD34 antibody (clone 8G12, BD Biosciences), and cell viability was estimated using propidium iodide (PI, BD Biosciences) by flow cytometry.

Reverse Transcription-Polymerase Chain Reaction for Detecting *SALL4* Expression

To examine the expression of *SALL4* gene at the mRNA level, reverse transcription-polymerase chain reaction was performed with cells harvested from the cell lines. The QuickPrep™ Micro mRNA purification kit (GE Healthcare, Boston, MA, USA) was used to extract mRNA. First-strand cDNA was synthesized with the use of bulk first-strand cDNA mix and random primer (GE Healthcare, Boston, MA, USA). PCR amplification using cDNA was performed according to the manufacturer's instructions (Qiagen, Boston, MA, USA). Oligonucleotides were synthesized as specific primers for *SALL4* by Integrated DNA Technologies Inc. (IA, Boston, MA, USA). Detection of β-actin was used as a control reaction in each run.

The sequences of primers were as follow: *SALL4* forward primer: 5′-CATGATGGCTTCCTTAGATGC CCCAG-3′, reverse primer: 5′-CCGTGTGTCATGT AGTGAACCTTTAAG-3′. β-Actin forward primer: 5′-GCACCCAGCACAATGAAG-3′, reverse primer: 5′-ACTCGTCATACTCCTGCTTG-3′.

The expected sizes of the PCR products were approximately 500 bp for *SALL4* and 120 bp for β-actin. PCR 50 bp low ladder (Sigma-Aldrich, St Louis, MO, USA) was run in parallel as a molecular size marker.

Immunostaining for *SALL4*

To examine the *SALL4* protein expression, immunostaining was performed according to standard techniques, which are briefly described here. Formalin-fixed, paraffin-embedded tissue sections of 5 μm thickness were de-paraffinized and hydrated. Heat-induced epitope was retrieved with the use of citrate buffer (pH 6; ZYMED Laboratories Inc., South San Francisco, CA, USA) and a pressure cooker (Biocare Medical, Concord, CA, USA). After incubation for 2 min at 100°C, slides were cooled for 25 min, rinsed in running tap water, and then washed for 5 min in 0.05 M Tris buffer (pH 7.4; Sigma-Aldrich) with 0.05% Tween-20 (DakoCytomation, Carpinteria, CA, USA). Endogenous peroxidase was then blocked by incubating the tissue sections in H_2O_2 for 5 min (DakoCytomation). Subsequently, the tissue sections were incubated for 1 h with the anti-*SALL4* antibody (1:500) in a humidified chamber at room temperature.[29] After three washes with Tris buffer, tissue sections were incubated with Envision anti-rabbit IgG (Dako-Cytomation) for 30 min at room temperature. After additional three washes, tissue sections were incubated with 250 μl of chromogen substrate (Dako-Cytomation) for 10 min or until color development. Hematoxylin (Fisher Scientific, Boston, MA, USA) was used as a counterstain. The immunostaining protocol for two fresh samples of hematopoietic stem/progenitor cells was essentially similar, except that the cytospin preparations were fixed in cold acetone (−20°C; Fisher Scientific) for 4 min at room temperature and then air-dried for 30 min prior to the immunostaining.

In each case, immunostaining was considered to be positive for *SALL4* when more than 15% of cells showed definitive nuclear staining. Acute myeloid leukemia was chosen as the positive control in this study on the basis of our previous survey of 81 acute myeloid leukemia cases; nuclear staining for *SALL4* protein was present in 100% of acute myeloid leukemia blasts.

Results

Detection of *SALL4* Protein Expression by Immunostaining in Benign and Neoplastic Hematopoietic Cells

Benign CD34$^+$ hematopoietic stem/progenitor cells, normal bone marrow aspirates, and leukemia samples
We first examined *SALL4* protein expression during normal hematopoietic differentiation (CD34$^+$

hematopoietic stem/progenitor cells and whole bone marrows), and its malignant counterpart, leukemia samples. As illustrated in Figure 1, immunostaining revealed that the *SALL4* protein was expressed in the nuclei of normal CD34$^+$ hematopoietic stem/progenitor cells ($N=2$) and those of precursor B-cell lymphoblastic leukemia/lymphomas blasts ($N=16$, 75%), and the staining pattern was similar to that observed in the positive control, acute myeloid leukemic blasts. In contrast, *SALL4* protein expression was negative in all precursor T-cell lymphoblastic leukemia/lymphomas ($N=12$, Figure 1d). Normal whole bone marrow trephine biopsy ($N=3$) samples were also negative for *SALL4* by immunohistochemistry. Positive cases were defined as >15% of immunostained cells with only nuclear staining. In normal whole bone marrow, <5% cells were observed to be positive, which were probably the CD34$^+$ hematopoietic stem/progenitor cells. Faint cytoplasmic staining was observed in occasional cases, but this was

regarded as nonspecific and only definitive nuclear staining was considered to be positive. Nonhematopoietic cells such as endothelial cells were negative.

Benign lymphoid tissues

We then studied *SALL4* protein expression in benign mature lymphoid tissues. By immunohistochemistry, *SALL4* protein expression was considered to be negative in 10 samples of normal lymphoid tissues examined, including two benign lymph nodes, four reactive tonsils, two benign thymus (surgically removed for cardiac operation), and two benign spleens (surgically removed due to trauma) (Figure 2). There were none or rare (<1%) positive cells detected in these samples.

Malignant lymphomas and plasma cell myeloma

We next investigated *SALL4* expression in the malignant counterpart of the benign lymphoid tissues, such as lymphoma and plasma cell myeloma. As illustrated in Figure 3 and summarized in
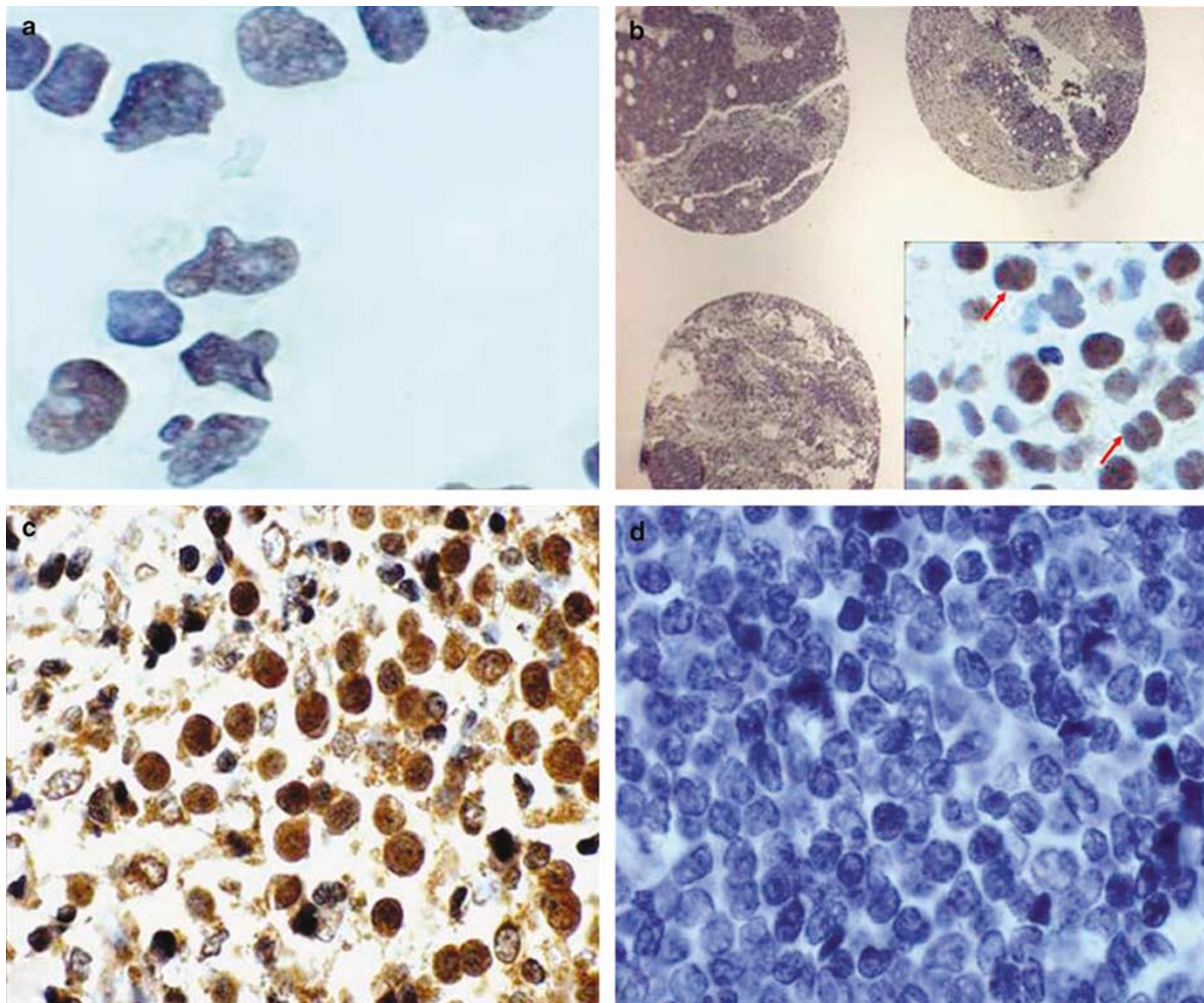

Figure 1 *SALL4* protein expression in normal CD34$^+$ hematopoietic stem/progenitor cells and lymphoblastic leukemia/lymphomas (immunoperoxidase). *SALL4* was positive in CD34$^+$ hematopoietic stem/progenitor cells (**a**, ×600) and B-cell lymphoblastic leukemia/lymphomas blasts (**c**, ×400), identical to the positive control (AML) (**b**, ×400), but negative in T-cell lymphoblastic leukemia/lymphomas (**d**, ×400).

Table 1, SALL4 protein expression was negative in all of the 113 B- or T-cell lymphoma and 32 plasma cell myeloma samples examined.

Detection of SALL4 mRNA Expression in Normal Peripheral Blood Mononuclear Cells, Benign Bone Marrow Aspirates, Acute Lymphoblastic Leukemia, and Hematopoietic Cancer Cell Lines

To correlate with the findings on its protein expression, we next examined the mRNA expression of SALL4 at different stages of normal hematopoietic differentiation, in primary leukemic samples and hematopoietic cancer cell lines. Summarized in Table 1 and illustrated in Figure 4, SALL4 mRNA was expressed in normal CD34$^+$ hematopoietic stem/progenitor cells ($N=3$, purity of normal CD34$^+$ hematopoietic stem/progenitor cells is 95, 95, and 98% with cell viability is 99, 99, and 97%, respectively, for each sample); it was down regulated in benign bone marrow aspirates ($N=2$) as SALL4 mRNA was barely detectable, probably due to its $<5\%$ components of CD34$^+$ hematopoietic stem/progenitor cells. No detectable mRNA expression for SALL4 was identified in normal peripheral blood ($N=5$). These results were consistent with our previous reports on SALL4 mRNA expression during normal hematopoiesis using real-time reverse transcription-polymerase chain reaction method.[29]
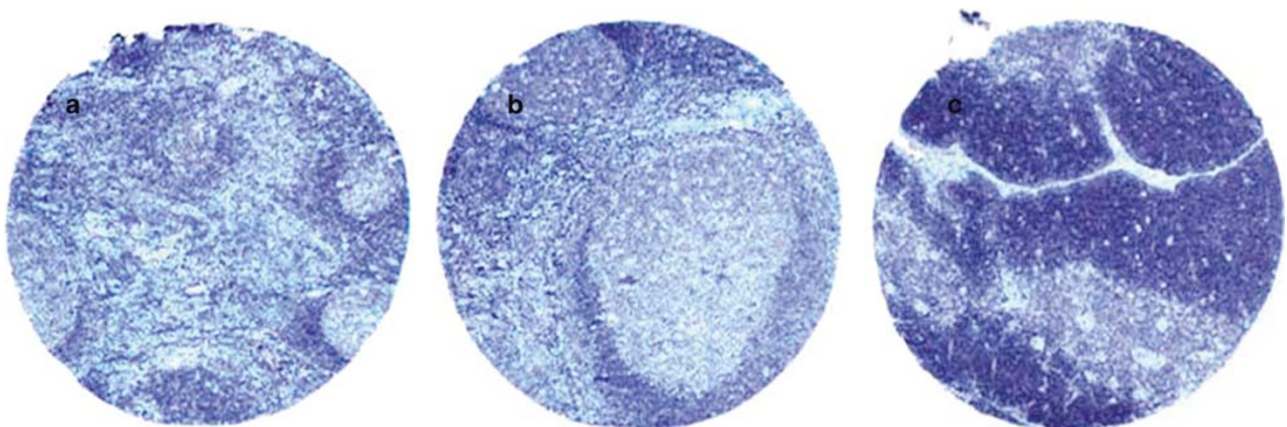
Figure 2 Immunohistochemical staining for SALL4 in normal lymph tissues. No detectable immunostaining (immunoperoxidase) of SALL4 protein was seen in benign lymph nodes (**a**, ×10), normal tonsil (**b**, ×10) and normal thymus (**c**, ×10). All staining of T zones, mantle zones, germinal centers, and splenic marginal zones was negative.
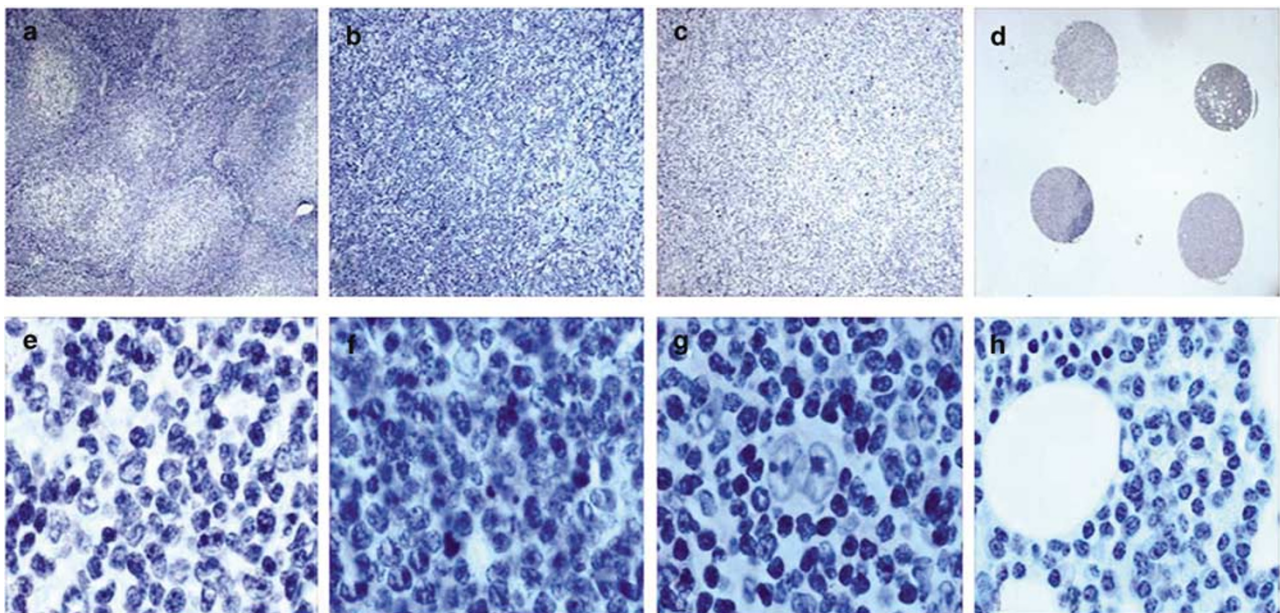
Figure 3 Immunohistochemical staining for SALL4 in lymphoma and plasma cell myeloma (immunoperoxidase). SALL4 protein was negative in follicular lymphoma (**a**, ×10; **e**, ×600), Burkitt's lymphoma (**b**, ×10; **f**, ×600), Hodgkin's lymphoma (**c**, ×10; **g**, ×600), and plasma cell myeloma (**d**, ×1; **h**, ×600).
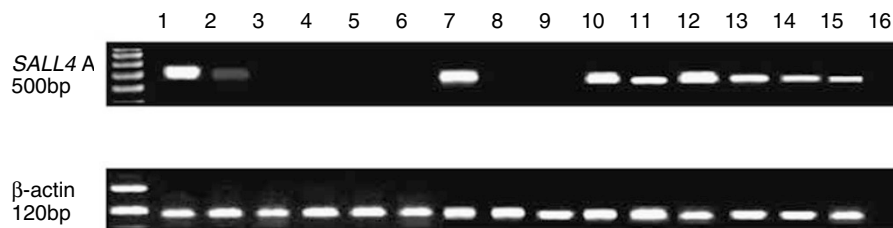
Table 1 *SALL4* expression in normal hematopoietic tissues, lymphoma, plasma cell myeloma and acute lymphoblastic leukemia

Primary tumor and normal tissue samples	Detection method (IHC, RT-PCR)	No. of SALL4-positive cases per total no. of cases examined (percentage)[a]
Non-Hodgkin's lymphoma	IHC	0/108
Hodgkin's lymphoma	IHC	0/5
Plasma cell myeloma	IHC	0/32
Precursor T-cell acute lymphoblastic leukemia/lymphoma	IHC	0/12
Precursor B-cell acute lymphoblastic leukemia/lymphoma	RT-PCR ($N=8$), IHC ($N=16$)	19/24 (79.1%)[b]
Positive control (AML)	IHC	5/5 (100%)
Normal CD34+ HSC/HPC cells	Immunocytochemistry ($N=2$); RT-PCR ($N=3$)	5/5 (100%)
Normal benign reactive lymphoid tissue	IHC	0/10
Normal bone marrow	Trepine biopsy assessed by IHC ($N=3$), aspirates assessed by RT-PCR ($N=2$)	0/5
Normal peripheral blood	RT-PCR ($N=5$)	0/5
Total		29/211 (13.7%)

RT-PCR = reverse transcriptase-polymerase chain reaction; IHC = immunohistochemistry.
[a]Criteria for positive expression of *SALL4* were the presence of only nuclear staining and staining of more than 15% of cells.
[b]In precursor B-cell lymphoblastic leukemia/lymphoma, seven of eight cases are positive for *SALL4* gene mRNA expression (7/8, 87.5%); 12 of 16 cases are positive for *SALL4* protein expression (12/16, 75%).

Figure 4 *SALL4* mRNA expression obtained from reverse transcription-polymerase chain reaction assay in normal hematopoietic tissues, primary B-cell lymphoblastic leukemia/lymphomas and cell lines from hematological malignancies. *SALL4* mRNA was detected in normal CD34+ hematopoietic stem/progenitor cells (lane 1), in precursor B-cell cell line Reh (lane 7), acute myeloid leukemia M2 cell line Kasumi (lane 10), and primary B-cell lymphoblastic leukemia/lymphomas bone marrow (lanes 11–15). *SALL4* mRNA was barely detected in normal whole bone marrow (lane 2). Negative results included: normal peripheral blood (lane 3), Burkitt's pre-B-cell lymphoma cell line BJAB (lane 4), B-cell lymphoblastic lymphoma cell line RL (lane 5), T-cell lymphoma cell line SUP-M2 (lane 6), T-cell lymphoblastic leukemia/lymphomas cell line JURKAT (lane 8), plasma cell myeloma cell line MMIS (lane 9), and negative control (lane 16). *β*-Actin was used as an internal control after 35 cycles.

Similar to our findings on *SALL4* protein expression, seven of eight B-cell lymphoblastic leukemia/lymphomas samples examined were positive for *SALL4*. Of the cell lines, only those of precursor B-cell lymphoblastic leukemia/lymphomas (Reh-1) and acute myeloid leukemia (Kasumi-1) were positive for *SALL4*. *SALL4* mRNA was negative in all of the lymphoma cell lines (RL, BJAB, and SUP-M2), those derived from the precursor T-cell lymphoblastic leukemia/lymphomas (JURKET), and plasma cell myeloma (MMIS).

Discussion

We recently identified that *SALL4*, a gene important for embryonic development, is constitutively expressed in human acute myeloid leukemia, and its overexpression in the transgenic mouse model leads to generation of acute myeloid leukemia.[29] These findings strongly implicate that *SALL4* is an oncogene which is involved in myeloid leukemogenesis. In order to further understand the biological significance of *SALL4*, particularly in the lymphoid tissues, we performed a survey on a broad range of normal and neoplastic hematopoietic cells. We found that the expression of the *SALL4* gene is restricted to the CD34+ hematopoietic stem/progenitor cells during normal hematopoiesis, and not in normal mature hematopoietic cells and benign reactive lymphoid tissues. Among the malignant counterparts, we only detected *SALL4* expression in acute myeloid leukemia and large portion of precursor B-cell lymphoblastic leukemia/lymphomas patient samples. Our studies of a series of hematopoietic cancer cell lines also revealed the same *SALL4* expression pattern, with *SALL4* expression detectable only in cell lines derived from acute myeloid leukemia and precursor B-cell lymphoblastic leukemia/lymphomas.

Like other *SALL* genes, *SALL4* plays an important role in human development, with heterozygous mutations of *SALL4* leading to Duane radial ray syndrome.[16,17,21] Its role in hematopoiesis is not completely understood. As revealed in this study, *SALL4* is only expressed in CD34^{+} hematopoietic stem/progenitor cells, but not mature myeloid or lymphoid cells. Thus, *SALL4* expression is down-regulated in normal maturation and differentiation in both myeloid and lymphoid cell lineages. This observation is in contrast with the expression of *SALL4* in most cases of acute myeloid leukemia and precursor B-cell lymphoblastic leukemia/lympho-mas, in which *SALL4* is constitutively expressed at the mRNA and protein levels. Thus, the failure of downregulation of *SALL4* in acute myeloid leuke-mia and precursor B-cell lymphoblastic leukemia/lymphomas correlates with the maturation arrest in the neoplastic blast population in these leukemias. We have already shown that the overexpression of *SALL4* in mice can lead to acute myeloid leukemia development in about 50% transgenic mice. In future studies, it will be of great interest to examine the remaining *SALL4* transgenic mice for the potential B-cell lymphoblastic leukemia/lympho-mas development.

While our findings suggest that *SALL4* might play roles in leukemogenesis of precursor B-cell lympho-blastic leukemia/lymphomas, this novel oncogene is not expressed in precursor T-cell lymphoblastic leukemia/lymphomas. These findings suggest that the pathogenesis of precursor B-cell lymphoblastic leukemia/lymphomas is different from that of pre-cursor T-cell lymphoblastic leukemia/lymphomas. Also in parallel with these findings, the vast majority of the chronic myeloid leukemia cases that transform into acute leukemia are rarely T-cell lymphoblastic leukemia/lymphomas, but rather acute myeloid leukemia or precursor B-cell lympho-blastic leukemia/lymphomas. These observations may be explained by a common pathway shared by the leukemogenesis of precursor B-cell lymphoblas-tic leukemia/lymphomas and acute myeloid leuke-mia. Recently, a B-cell/myeloid common progenitor that gives rise to both B and myeloid lineages has been identified as a population CD34^{+}CD19^{+} CXCR4^{−} in humans and c-Kit^{+}B220^{+}CD19^{−} in mice.[30,31] Additional evidence supporting the ex-istence of a B-cell/myeloid common progenitor comes from a functional study of the transcription factor *PU.1* in hematopoiesis.[32,33] When *PU.1* was homozygously deleted in a murine model, no B cells, macrophages, or granulocytes could be de-tected, and the animals were anemic.[32] One expla-nation for this observed phenotype in *PU.1*-knockout mice is that *PU.1* is essential to the differentiation of B-cell and myeloid lineages, but not that of T-cells. Therefore, on the basis of *SALL4*'s similar expression patterns in acute myeloid leuke-mia and B-cell lymphoblastic leukemia/lymphomas, we hypothesize that *SALL4* (in analogy to *PU.1*) is required for the development of the B-cell/myeloid common progenitor, and dysregulation of its expres-sion can lead to maturation arrest of these cells and predisposing to leukemogenesis of acute myeloid leukemia and precursor B-cell lymphoblastic leuke-mia/lymphomas. While the precise stage position of B-cell/myeloid common progenitor population needs further definition within the hematopoietic paradigm, the identification of these B/myeloid common progenitors support the concept that the development of the B-cell and the myeloid lineages is more closely related than that of either cell type with the T-cell development.

While our data may implicate the role of *SALL4* in leukemogenesis of precursor B-cell lymphoblastic leukemia/lymphomas, a small subset of these cases had no evidence of *SALL4* expression, either at the mRNA or protein levels. These findings suggest the pathogenesis of precursor B-cell lymphoblastic leukemia/lymphomas is heterogeneous. Larger stu-dies with more cases will be required to compare *SALL4*-positive and *SALL4*-negative cases, in terms of morphology, cytogenetics, patient age, and clin-ical outcome.

Lastly, our data suggest that *SALL4* may be diagnostically useful due to its high specificity to acute myeloid leukemia and precursor B-cell lym-phoblastic leukemia/lymphomas. Similar to term-inal deoxynucleotidase, *SALL4* appears to be a marker for immaturity and thus is useful in distinguishing precursor B-cell lymphoblastic leu-kemia/lymphomas from other aggressive B-cell lymphoma such as Burkitt's lymphoma.

Acknowledgements

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1592

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