# Expression of the polycomb group gene *Bmi*1 does not affect the prognosis of pediatric acute lymphoblastic leukemia

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## ABSTRACT

**The Polycomb group protein *Bmi*1 is a constituent of the Polycomb repressive complex 1, and it is an important molecule for the regulation of the self-renewal of hematopoietic stem cells. In the field of clinical hematology, there are reports that the level of *Bmi*1 expression in blast cells is related to the prognosis of acute myeloid leukemia, chronic myeloid leukemia, and myelodysplastic syndrome. We investigated whether the level of *Bmi*1 expression in leukemic cells is related to the prognosis and the characteristics of childhood acute lymphoblastic leukemia. In all the leukemic blast cells, *Bmi*1 gene expression was lower value than that in normal B cells. There were no correlations between the level of *Bmi*1 gene expression in leukemic blast cells and other parameters, including prognosis. Here, we report that the level of *Bmi*1 expression in blast cells is not related to the prognosis of pediatric acute lymphoblastic leukemia.**

**Keywords:** Acute Lymphoblastic Leukemia; Leukemic Stem Cell; *Bmi*1; Polycomb

## 1. INTRODUCTION

Mammalian Polycomb group (PcG) protein complexes can be classified into 2 distinct types, Polycomb repressive complex 1 (PRC1) and PRC2. The PcG protein *Bmi*1 is a constituent of PRC1 [1-5]. *Bmi*1 is an important molecule for the self-renewal of hematopoietic stem cells (HSCs). The expression of *Bmi*1 is generally high in HSCs but decreases after these cells differentiate [6,7]. Some reports in the field of clinical hematology have shown that the expression level of *Bmi*1 in blast cells of acute myeloid leukemia (AML), chronic myeloid leukemia (CML), and myelodysplastic syndrome (MDS) can

be related to the prognosis of the disease [8-10]. According to these reports, leukemia cells that have a high *Bmi*1 expression are correlated with poor prognosis. Cancer stem cells in AML were identified for the first time in 1997. In that study, CD38–/CD34+ cells together with leukemic cells were injected into non-obese diabetic mice with severe combined immunodeficiency disease, and the injected mice developed AML [11]. Subsequently, stem cells of breast cancer, brain tumor, prostate cancer, colon cancer, and pancreatic cancer were successively reported [12-16]. Although there are reports on the stem cells of acute lymphoblastic leukemia, these are fewer than those on other cancers [17,18]. A high expression of *Bmi*1, which has been reported for many cancer stem cells, might be the reason for some cancers becoming chemoresistant [19,20]. We investigated whether *Bmi*1 expression is related to the prognosis and cell properties of pediatric acute lymphoblastic leukemia.

## 2. MATERIALS AND METHODS

### 2.1. Patients

Because childhood leukemia is usually precursor B leukemia, we limited our investigation to pediatric precursor B acute lymphoblastic leukemia. According to the Declaration of Helsinki, informed consent and permission were obtained from all patients. Leukemic blast cells were collected from patients who had been diagnosed with pediatric precursor B acute lymphoblastic leukemia at Hiroshima university hospital from January 2002 to September 2006. These leukemic blast cells were used for real-time polymerase chain reaction (RT-PCR). Cells were obtained from only those patients in whom the percentage of leukemic cells was higher than 90%, and only CD19+ cells were chosen.

### 2.2. Flow Cytometric Cell Sorting

The bone marrow-derived cells were labeled with dif-

ferent antibodies to sort each hematopoietic lineage. CD34+/CD38−, CD34+/CD38−/CD10−, CD34+/CD38+/ CD10+, CD3+, and CD19+ cells were treated as HSCs, common myeloid progenitors, common lymphoid progenitors, T cells, and B cells, respectively. Dead cells that positively stained with propidium iodide were excluded. The cells were sorted using a FACS Aria system (BD Biosciences, Bedford, MA). Sorted cells with a purity of at least 95% were used for further experimentation.

### 2.3. Real Time RT-PCR

The TaqMan RT-PCR was used for quantitative gene analysis (TaqMan PCR; Applied Biosystems, Foster City, CA). The sequences of the specific primers were as follows: *Bmi*1, sense primer: 5'-AAA TGC ATC GAA CAA CGA GAA TC-3' and antisense primer: 5'-AAT GAA GTA CCC TCC ACA AAG CA-3'; *GAPDH*, sense primer: 5'-GAA GGT GAA GGT CGG AGT-3' and antisense primer: 5'-CTT TAG GGT AGT GGT AGA AG-3'. The sequences of the 5'-FAM3'-TAMRA labeled probes were as follows: *Bmi*1, 5'-CAG GTG GGG ATT TAG CTC AG-3' and *GAPDH*, 5'-CCG ACT CTT GCC CTT CGA AC-3'. The relative quantification (ΔΔCT method) was used for quantitative analysis.

### 2.4. Statistical Analysis

The correlation coefficient was analyzed with Pearson

product-moment correlation coefficient. Welch's t test was used for analyzed between 2 groups.

## 3. RESULTS

Firstly, we purified the cell populations of each differentiation stage, including the HSCs from the bone marrow of healthy volunteers and analyzed the expression of the *Bmi*1 gene. The expression of the *Bmi*1 gene is high in HSCs, and decreases when the cells enter the differentiation stage, as shown **Figure 1(a)**. This result is in agreement with a previous report [21]. The inhibition of T-cell differentiation has been reported in *Bmi*1-deficient mice [22]. Therefore, it is thought that *Bmi*1 is essential for T-cell maturation. The expression of the *Bmi*1 gene was high in T cells in moderation. The expression of the *Bmi*1 gene did not increase when B cells underwent differentiation. These results are summarized in the schema (**Figure 1(b)**). The expression of *Bmi*1 in HSCs is 1.0. The expression of the *Bmi*1 gene was lower than HSCs at all hematopoietic stages. In other words, the expression of the *Bmi*1 gene decreased during the differentiation of the hematopoietic cells. In addition, it seems that undifferentiated cells have a high level of *Bmi*1 expression.

**Table 1** summarizes the basic data of all participants such as the peripheral blood white blood cell count, cell properties, and age. Unfortunately, 4 patients (Nos. 12 - 14)

**Table 1.** Summary of the patients' profiles, cell profiles, and the level of *Bmi*1 expression.

| case | age | sex | WBC counts in PB at the onset (/mm³) | risk | *Bmi*1/*GAPDH* | positive for [percentage (%)] | | | | | | hospitalization days | |
|------|-----|-----|------|------|------|------|------|------|------|------|------|------|------|
| | | | | | | CD10 | CD19 | CD20 | CD33 | CD34* | CD38 | | |
| 1 | 2y8m | M | 9400 | standard risk | 0.241 | 88.6 | 88.2 | 1.8 | 1.6 | 86.8 | 33.5 | 144 | |
| 2 | 3y1m | M | 6900 | high risk | 0.430 | 56.2 | 97.6 | 25.4 | 0.3 | 24.4 | 99.1 | 334 | |
| 3 | 6y2m | F | 30,910 | high risk | 0.057 | 78.4 | 96.5 | 22.0 | 0.9 | 93.1 | 76.4 | 203 | |
| 4 | 0y9m | F | 275,400 | infant | 0.076 | 89.5 | 92.7 | 8.1 | 0.8 | 84.2 | 69.1 | 436 | relapse |
| 5 | 4y10m | F | 5200 | standard risk | 1.253 | 91.5 | 94.1 | 1.4 | 56.9 | 8.9 | 85.7 | 166 | |
| 6 | 7y5m | M | 9290 | high risk | 1.683 | 45.4 | 79.3 | 80.7 | 2.3 | 0.6 | 91.5 | 173 | |
| 7 | 9y2m | M | 39,320 | high risk | 0.127 | 86.7 | 89.3 | 4.3 | 0.9 | 61.8 | 95.8 | 187 | |
| 8 | 5y5m | F | 7920 | bcr-abl + | 0.040 | 81.0 | 84.4 | 70.8 | 29.2 | 59.9 | 73.8 | 243 | |
| 9 | 6y0m | F | 7600 | high risk | 0.050 | 79.6 | 76.2 | 21.1 | 2.6 | 2.0 | 98.4 | 236 | |
| 10 | 1y6m | M | 4500 | standard risk | 0.567 | 90.9 | 91.4 | 62.4 | 3.1 | 90.6 | 82.8 | 139 | |
| 11 | 8y3m | F | 4210 | standard risk | 1.023 | 86.6 | 89.1 | 18.6 | 2.7 | 14.9 | 91.7 | 118 | |
| †12 | 7y3m | F | 4900 | high risk | 0.063 | 70.7 | 95.2 | 4.0 | 0.4 | 94.5 | 98.3 | 529 | relapse |
| †13 | 0y1m | F | 25,340 | infant | 0.065 | 1.5 | 89.8 | 2.4 | 23.1 | 92.0 | 99.2 | 86 | |
| †14 | 6y9m | F | 278,000 | bcr-abl + | 0.153 | 91.8 | 91.4 | 44.6 | 0.6 | 25.2 | 80.7 | 364 | |
| †15 | 1y7m | F | 59,770 | high risk | 0.272 | 2.0 | 96.9 | 3.3 | 0.4 | 1.3 | 91.7 | 443 | |

†death case; *p < 0.05.
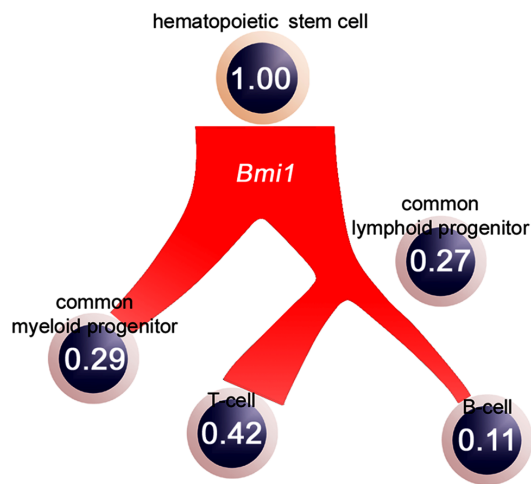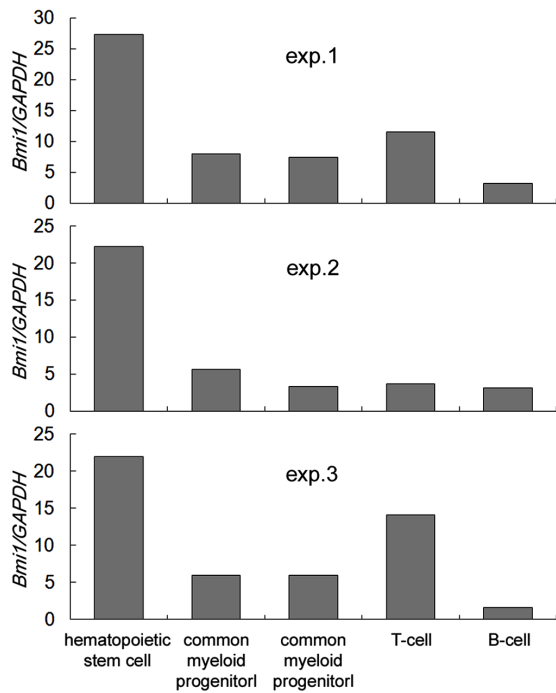
**Figure 1.** Quantitative analysis of *Bmi*1 gene expression of each hematopoietic cell type. (a) CD34+/CD38–, CD34+/ CD38+/CD10–, CD34+/CD38+/CD10 +, CD3+, and CD19+ cells were treated as HSCs, common myeloid progenitors, common lymphoid progenitors, T cells, and B cells, respectively. The expression of *Bmi*1 increased in immature cells; (b) *Bmi*1 gene expression in each lineage are shown in the schema. The expression of the *Bmi*1 gene decreased during the differentiation of hematopoietic cells.

died in spite of treatment. **Figure 2(a)** compares *Bmi*1 gene expression in the blast cells and B cells (CD19+ cells) from the bone marrow of healthy donors. *Bmi*1 gene expression in all blast cells was low compared to normal B cells. There was no correlation between the prognosis of the patients and the level of *Bmi*1 gene ex-
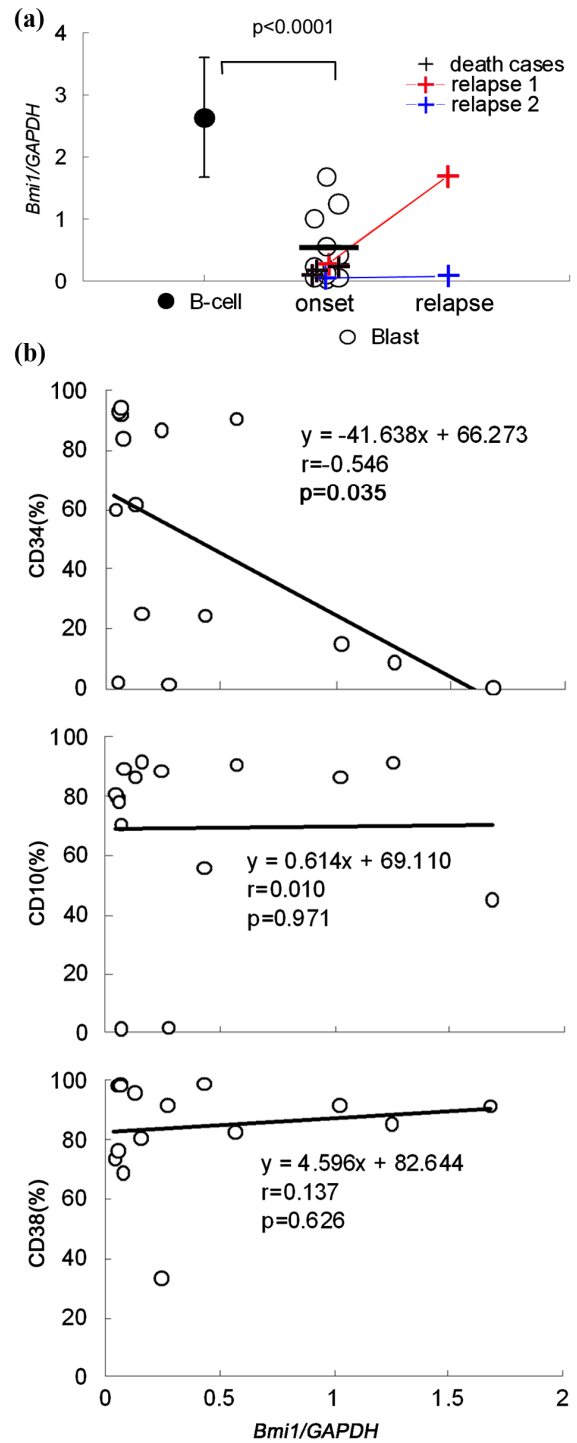


**Figure 2.** Comparison of the level of *Bmi*1 expression in normal B cells and precursor B cells of acute lymphoblastic leukemia. (a) The level of *Bmi*1 expression is significantly decreased in all leukemic cells as compared to normal B cells. The level of *Bmi*1 expression was not significantly different between recurrent cases and death cases. Error bars indicate standard deviation; (b) There was a correlation between *Bmi*1 gene expression and the cell surface marker CD34. There was no significant difference between other surface markers.

pression. The expression of the *Bmi*1 gene was analyzed using cells from the bone marrow at the time of recurrence in 2 cases (of 4 relapse cases). In one of these 2 cases, *Bmi*1 expression in the blast cells increased from the first onset but did not change in the second case. However, both patients had low expression levels compared to normal B cells. There was a negative correlation between *Bmi*1 gene expression and the cell surface marker CD34 (**Figure 2(b)**). It is known that human HSCs harbor the CD34 antigen. Therefore, we expected that CD34+ cells are undifferentiated cells and express *Bmi*1. However, this result was contrary to our expectation.

## 4. DISCUSSION

We investigated whether the level of *Bmi*1 gene expression in pediatric precursor B leukemic cells is related to the clinical course, cell profile, and prognosis. There are reports on a relationship between the prognosis of hematopoietic malignancies and *Bmi*1 gene expression. Especially in AML, CML, and MDS, when the expression of the *Bmi*1 gene increased in leukemic blasts, the prognosis was bad [8-10]. Yet, the reason for this is not known. However, the *Bmi*1 protein regulates the p16INK4a protein, which is one of the CDK (cylcin-dependent kinase) inhibitors [23]. In addition, the proliferation of leukemic cells may increase due to *Bmi*1 expression. *Bmi*1 is an essential molecule for the self-renewal of HSCs. It has been reported that the selfrenewal of HSCs reduces remarkably in *Bmi*1-deficient mice [7]. Immature cells such as HSCs have a high expression of *Bmi*1, and *Bmi*1 expression decreases in developed cells during differentiation [21,24]. It has been reported that the expression of the *Bmi*1 gene is high in side population cells such as cancer stem cells in heaptocellular carcinoma [25]. It can be expected that the expression of the *Bmi*1 gene increases in cancer stem cells according to these previous studies. Usually, cancer stem cells are chemoresistant. In addition, it was shown that cancer stem cells are involved in cancer recurrence [26-28]. A high expression of *Bmi*1, which has been reported for many cancer stem cells, might be the reason for some cancers becoming chemoresistant [19,20]. Therefore, there is the possibility that the cancer cells are not exterminated, and the prognosis of the patient worsens. In acute lymphoblastic leukemia, it was reported that CD90+/CD110+ cells in acute lymphoblastic T-cell leukemia, and CD9-overexpressing cells in acute lymphoblastic B-cell leukemia, are cancer stem cells [17,18]. Although these data were collected from in vitro experiments, the existence of cancer stem cells in primary precursor B acute lymphoblastic leukemia cells and pediatric acute lymphoblastic leukemia cells is not clarified yet. In our data, there was the significance of the negative

correlation between CD34 and *Bmi*1 expression of blast cells. Generally, it is known that human CD34 positive cells are immature cells. When there are many immature cells, poor prognosis was expected. There were a few reports that CD34 is a prognostic factor in adults with acute myelocytic leukemia [29,30]. However, there were no reports showing the relation between CD34 and prognosis of acute lymphoblastic leukemia. Both CD34 and the expression of *Bmi*1 were not correlated with prognosis in our data. *Bmi*1 expression in pediatric acute lymphoblastic leukemia cells is lower than that in normal B cells. We do not know why the *Bmi*1 expression in pediatric acute lymphoblastic leukemia cells is lower than *Bmi*1 expression in B-cells. Although it is our speculation, pediatric acute lymphoblastic leukemia cells are no longer in immature. Moreover, cancer stem cells in pediatric acute acute lymphoblastic leukemia may be nonexistent.

## 5. CONCLUSION

We investigated whether the level of *Bmi*1 expression in leukemic cells is related to the prognosis and the characteristics of childhood acute lymphoblastic leukemia. From this result, it will be a very small number even if immature cells like cancer stem cells exist. In addition, there is no correlation between *Bmi*1 expression and the prognosis of pediatric acute lymphoblastic leukemia. Cancer stem cells may not exist in pediatric acute lymphoblastic leukemia. However, the number of people participating in the experiment is not enough in this study. We consider the samples from more patients with leukemia will be necessary to lead certain conclusion.

## 6. ACKNOWLEDGEMENTS

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