Dear Editor:

AML1-ETO fusion transcription factor is one of the most frequent chromosomal abnormalities detected in human acute myeloid leukaemia subtype 2 (M2). However, AML1-ETO alone is insufficient to develop leukaemia [1, 2]. To search for putative novel leukaemia associated genes, we therefore studied the gene expression profiles in AML-M2. BAALC as one of the genes found may be crucial in the pathogenesis of AML-M2. Some studies have reported that overexpression of BAALC gene was seen in patients with AML and ALL, and more strikingly, in a subset of AML with normal karyotype while predicting a poor prognosis [3–9]. However, its importance in AML-M2 patients with t (8; 21) translocation had never been mentioned.

To explore the function of BAALC and its possible role in the pathogenesis of human myeloid leukaemia subtype 2 (M2) in a hope of finding one more useful biomarker for molecular diagnosis, we have examined the expression level of BAALC in bone marrow mononuclear cells (MNCs) of patients with M2 by using quantitative real-time RT-PCR. Compared to 15 samples of normal bone marrow cells, the expression level of BAALC was significantly up-regulated in 18 pretreated M2 patients. The median level of BAALC transcripts in pretreated patients with AML-M2 and non-malignant blood diseases was 3.93 (ranging 0.71–8.94) and 0.04 (ranging 0.01–0.14), respectively (Fig. 1). The abundance of BAALC mRNA relative to that of ABL mRNA in the cells from most M2 patients was significantly greater than that in the MNCs from non-malignant blood diseases (P < 0.001) (Fig. 1, Table 3).

Results from 61 samples of M2 patients at different stages showed that the expression pattern of BAALC was generally parallel to that of AML1-ETO fusion gene in AML-M2 patients with t(8;21) translocation. To evaluate the impact of BAALC expression values on clinical outcome without seeking an optimal cut point, the expanded cohort of AML patients were dichotomized at the median value and divided into two expression groups. In high BAALC group, samples appeared to synthesize more copies of AML-ETO transcript (n = 21; median expression = 9.66, range 0.5–27.5), compared with low BAALC group (n = 40; median expression = 0.45, range 0–12.1). The percentage of myeloid blast cells in bone marrow cells in high BAALC group was markedly higher than that in low BAALC group, namely 53.2% (n = 12) versus 7% (n = 26). In addition, the serum levels of lactic dehydrogenase (LDH) were also significantly different between these two groups. High level of LDH was 698.7 (n = 11) in high BAALC group versus 194.6 (n = 25) in low BAALC group (Fig. 2).

Besides, for each individual patient, BAALC was expressed at a high level, at pretreated stage, while became lower in partial remission or complete remission. In 6 patients, the expression of BAALC was detected high in pretreated phase, and turned lower during remission phase. The same tendency was found in the expression of AML1-ETO (Fig. 3).

In all patients, we have compared the expression level of BAALC among different clinical outcome groups, demonstrating significant differences (Table 1). In overall samples, the expression level of BAALC was positively correlated with the AML1-ETO transcription level (r = 0.63, P < 0.001). A positive correlation between BAALC expression level and the percentage of myeloid blasts (r = 0.68, P < 0.001), or LDH level (r = 0.39, P < 0.05) in the serum was also detected (Table 2).

Our present study has shown that the expression pattern of BAALC was generally parallel to AML1-ETO fusion gene in AML-M2 patients with t(8;21) translocation. The high BAALC group patients contained more myeloid blast cells in bone marrow, and had higher level of LDH in serum in comparison with low BAALC group. Besides, the high level of BAALC expression in patients at pretreated stage remarkably reduced to a significant low level after achievement of complete remission. Taking all these together, our results suggested that the AML-M2 patients in high BAALC expression group suffered a higher malignant burden and may have a poor prognosis [9]. Some reports showed that allogeneic haematopoietic stem cell transplantation might overcome the adverse prognostic effect of high BAALC expression [9]. This may assist to design a personal therapeutic plan in advance for those who expressed high level of BAALC at diagnosis.

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doi:10.1111/j.1582-4934.2008.00447.x
Quantification of BAALC mRNAs in the BMNCs bone marrow samples from patients with AML-M2, or non-malignant blood disease. The cDNA prepared from the bone marrow cells of 33 patients, consisting of 18 pretreated AML-M2 cases, and 15 with non-malignant blood disease as controls. These cDNAs were subjected to real-time quantitative RT-PCR analysis with primers specific for BAALC and ABL. The ratio of the abundance of BAALC transcripts to that of ABL transcripts (BAALC/ABL) was calculated for statistical analysis. (**P < 0.001)

Statistical analysis on AML1-ETO expression, percentage of myeloid blast in BM and serum level of LDH in AML-M2 patients with different BAALC expression. AML patients with high BAALC expression show significantly higher AML1-ETO (A), percentage of myeloid blast (B), and serum LDH (C) compared to low BAALC patients (**P < 0.01, *P < 0.05).
Fig. 3 Correlation between BAALC expression and clinical phase BAALC were detected in pretreated phase and remission phase in 6 patients. AML-ETO was detected at the same time.

Table 1 Comparison of BAALC expression during different clinical stages

| Clinical outcome         | BAALC<sup>a</sup> | AML-ETO<sup>b</sup> | BLAST<sup>c</sup> | LDH<sup>d</sup> |
|--------------------------|-------------------|---------------------|-------------------|-----------------|
|                          | $\overline{x} \pm SD(n)$ | $\overline{x} \pm SD(n)$ | $\overline{x} \pm SD(n)$ | $\overline{x} \pm SD(n)$ |
| Pretreated stage         | 3.93 ± 2.15(18)   | 9.8904 ± 7.7009(18) | 0.565 ± 0.265(12)  | 707.73 ± 563.5(11) |
| After in remission       | 0.68 ± 1.03(36)   | 0.0488 ± 1.5167(36) | 0.059 ± 0.062(27)  | 189.58 ± 88.00(24) |

<sup>a</sup> $t' = 6.07$, $P < 0.0001$; <sup>b</sup> $t' = 5.13$, $P < 0.0001$; <sup>c</sup> $t' = 6.54$, $P < 0.0001$; <sup>d</sup> $t' = 6.07$, $P = 0.0123$.

Table 2 Correlation coefficient among the BAALC, AML-ETO, Blast percentage and LDH level

|          | BAALC  | AML-ETO | BLAST | LDH  |
|----------|--------|---------|-------|------|
| BAALC    | 1.000  | 0.6336*** | 0.6769*** | 0.3947* |
| AML-ETO  | 1.000  | 0.6820*** | 0.4458*  |      |
| BLAST    | 1.000  | 0.7670*  |       |      |
| LDH      | 1.000  |         |       |      |

* $P < 0.05$, ***$P < 0.0001$. 
Table 3 Sequences of primers and TaqMan probes for BAALC, AML1-ETO and ABL

|    | F                                      | R                                      | Probe                                  |
|----|----------------------------------------|----------------------------------------|----------------------------------------|
| AML-ETO | 5′-CACAGAGCCATCAAATCACAGT-3′            | 5′-GCATTGTGAGTGCTTCTCAGTA-3′           | 5′-FAM-ATGGGCCCGAGAACCTCGAAG-3′       |
| ABL  | 5′-GATACGAGGAGGGTGTACCA-3′             | 5′-CTCAGCGGTGGTTGAA-3′                 | 5′-FAM-AGCGTAAGGGGAGATTGCTTCA-3′      |
| BAALC | 5′-GCCAATGCAATCAAATAAGGA-3′            | 5′-AAGCAAGGAACTGAAGAACC-3′             | 5′-FAM-ACCCCTAGGCTACGATGAG-3′        |

Acknowledgements

This work is supported by grant #30470733 from The National Natural Scientific Foundation of China. Grant #BK2004040 from the Natural Scientific Foundation of Jiangsu Provincial Scientific Bureau of China. Grant #135XY0407 from 135 open grant of Jiangsu Provincial Scientific Bureau of China.

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