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Up-regulation of *BAALC* gene may be an important alteration in AML-M2 patients with t(8;21) translocation

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 were 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 diagnose.

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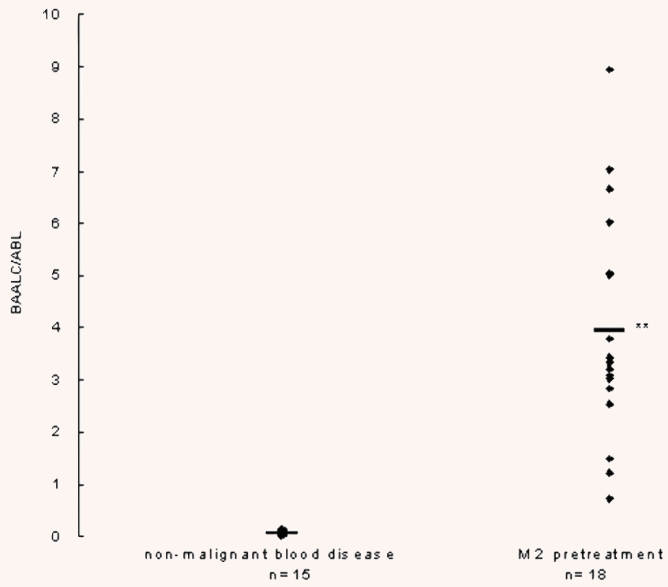

Fig. 1 Quantification of *BAALC* mRNAs in the BMNCs bone marrow samples from patients with AML-M2, or non-malignant blood disease. The cDNA prepared from the bone marrow cells of 33 patients, consisting of 18 pretreated AML-M2 cases, and 15 with non-malignant blood disease as controls. These cDNAs were 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*)

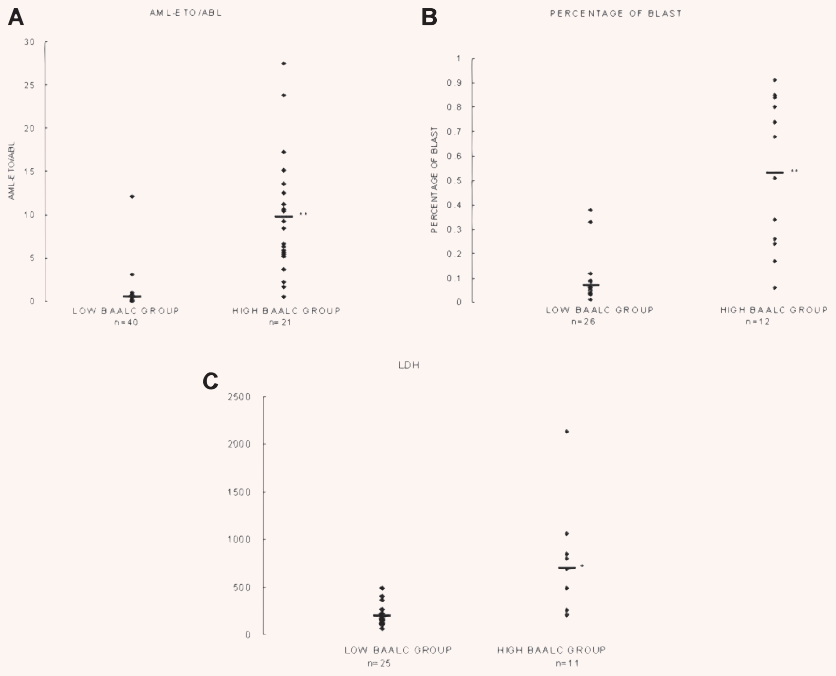

Fig. 2 Statistical analysis on *AML1-ETO* expression, percentage of myeloid blast in BM and serum level of *LDH* in AML-M2 patients with different *BAALC* expression. AML patients with high *BAALC* expression show significantly higher *AML1-ETO* (**A**), percentage of myeloid blast (**B**), and serum *LDH* (**C**) compared to low *BAALC* patients (***P < 0.01, *P < 0.05*).

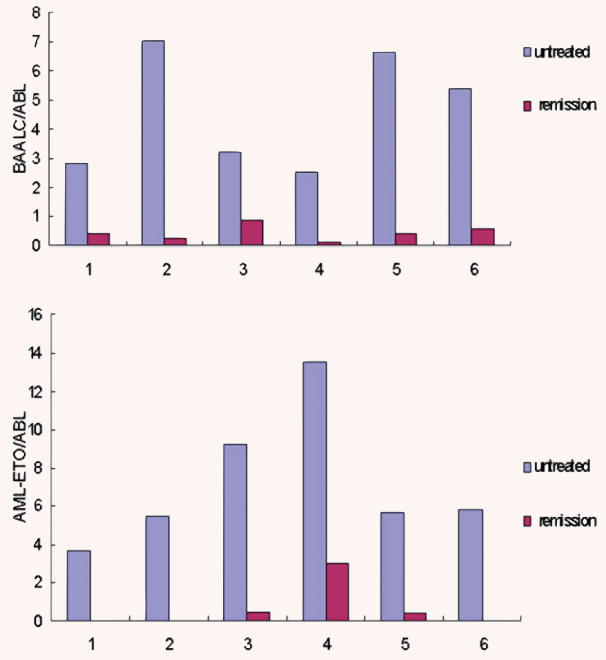

Fig. 3 Correlation between *BAALC* expression and clinical phase *BAALC* were detected in pretreated phase and remission phase in 6 patients. *AML-ETO* was detected at the same time.

Table 1 Comparison of *BAALC* expression during different clinical stages

Clinical outcome	*BAALC*[a]	*AML-ETO*[b]	BLAST[c]	*LDH*[d]
	$\overline{X} \pm SD(n)$	$\overline{X} \pm SD(n)$	$\overline{X} \pm SD(n)$	$\overline{X} \pm SD(n)$
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)

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

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 TagMan probes for *BAALC*, AML1-ETO and ABL

	F	R	Probe
AML-ETO	5′-CACAGAGCCATCAAAATCACAGT –3′	5′-GCATTGTGGAGTGCTTCTCAGTA-3′	5′-FAM-ATGGGCCCCGAGAACCTC-GAA -TAMRA-3′
ABL	5′-GAT ACG AAG GGA GGG TGT ACC A-3′	5′-CTC GGC CAG GGT GTT GAA-3′	5′-FAM -TGCTTCTGATGGCAAGCTC-TACGTCTCCT- TAMRA-3′
BAALC	5′-GCCAATGCATCCAAATAAGGA -3′	5′-AAGCAAAGGGAACTGGAAAGC-3′	5′-FAM – ACCCCTCAGGGCTCAGC-TAGACATTGC- TAMRA-3′

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