Asia Pacific Journal of Molecular Biology and Biotechnology, 2004
Vol. 12 (1&2) : 49-52

The Detection of *AML1/ETO* Fusion Transcript in Acute Myeloid Leukaemia in Universiti Sains Malaysia Hospital

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Received 12 April 2004 / Accepted 20 November 2004

Abstract. The translocation (8;21)(q22;22) is one of the most common chromosomal aberrations seen in patients with acute myeloid leukaemia (AML), occuring in the frequency of 7 to 12 % of cases . The t(8; 21) results in the formation of a chimeric *AML1/ETO* transcript. The aim of this study was to detect *AML1/ETO* fusion transcript in patients with AML diagnosed in Universiti Sains Malaysia Hospital. RNA from 24 whole blood samples were extracted from these patients and subjected to RT-PCR using specific primers for *AML1* and *ETO* genes. Four of these patients (16.7%) were found to have *AML1/ETO* fusion transcript. Morphologically 3 of them were classified as AML-M2 (FAB classification) and 1 was classified as AML-M1. Only one of those positive samples was sent for cytogenetic analysis and was found to have t (8;21). All 3 patients with AML-M2 had aberrant expression of CD19. Thus, RT-PCR detection of *AML1/ETO* may identify a subgroup of AML patients who carry a better prognosis.

Keywords: **acute myeloid leukaemia, *AML1/ETO*, RT-PCR**

INTRODUCTION

The t(8;21) translocation is one of the most common abnormalities found in acute myeloid leukaemia. It is found in approximately 10-15% of all AML cases and 30-40% of AML-M2 (Downing,1999) according to the FAB classification (Belnet *et al.*, 1985; 1988; 1976). Although more than 90% of t(8;21) are found to be associated with AML-M2, this cytogenetic abnormality is also found in approximately 6% of AML-M1, rarely in AML-M4 and other myeloproliferative diseases (Langabeer *et al.,* 1997; Wong *et al.,* 1993; de Greef and Hagemeijer., 1996). Immunophenotypic analysis done on blast cells with t(8;21) showed that there is frequent expression of CD19 and higher levels of CD34 and HLA-DR expression.

The t(8;21) was cloned in 1991 (Kawano *et al.,* 1997). Two genes are involved in this abnormality, the *AML*1 *gene* on chromosome 21q22 and the *ETO gene* on chromosome 8q22 (Downing, 1999; Kawano *et al.,*1997). This translocation results in the formation of a chimeric *AML*1/*ETO* transcript (Kawano *et al.,*1997; Maruyama *et al.,*1993). The product of *AML*1/*ETO* plays a critical role in the pathogenesis of AML where it results in an increased in haemopoietic cell proliferation with high self renewal capacity (Downing,1999; Barragan *et al.,*1998). Its presence in AML generally confers relatively favorable prognosis (Mrozek *et al.,*2001). In addition, it is a good target for the detection of minimal residual disease and monitoring of therapy (Maruyama *et al.,*1993; van Dongen *et al.*, 1999).

In our institution, there is no study done as yet to detect the presence of t(8;21) in AML patients. Thus the objective of our study was to detect the *AML*1/*ETO* fusion transcript in patients with AML and to determine its relation with the FAB subtypes and immunological markers.

MATERIALS AND METHODS

Sample Preparation. Bone marrow and peripheral blood samples were obtained from 24 patients with AML. Morphological diagnoses and classification were based on analysis of peripheral blood and bone marrow

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Table 1. Clinical and laboratory features of patients diagnosed as acute myeloid leukaemia

No. of pts	Age (Yr)	Sex	Hb (g/l)	TWBC ($x10^9/l$)	Plt ($x10^9/l$)	FAB	Monoclonal antibodies				RT-PCR
							CD13	CD33	CD19	HLA-DR	
1	10	M	99	4.3	37	1	ND	ND	ND	ND	+
2	34	F	78	21.9	17	2	+	+	+	+	+
3	5	F	109	27.8	106	BIL	+	+	+	+	-
4	31	M	75	26	8	1	+	+	-	ND	-
5	31	M	117	393.5	45	BIL	+	-	-	ND	-
6	57	F	64	0.8	25	3	ND	ND	ND	ND	-
7	5	M	76	8.3	69	7	+	+	-	+	-
8	54	M	89	39.7	95	2	+	+	-	_	-
9	9	M	66	91.2	160	4	+	+	-	+	-
10	13	M	23	4.8	11	2	ND	ND	ND	ND	-
11	18	M	82	7.8	17	7	-	-	+	+	-
12	18	F	73	17.4	30	3	+	+	-	ND	-
13	13	M	111	22.4	5	2	+	+	+	+	+
14	65	F	53	55.7	28	3	+	+	-	ND	-
15	33	F	81	6.2	170	2	+	+	-	ND	+
16	45	M	71	3.8	37	3	+	+	+	+	-
17	32	F	72	280.1	40	BIL	+	+	+	+	-
18	60	F	118	3.8	19	4	-	+	-	+	-
19	3	F	59	83	29	2	ND	ND	ND	ND	-
20	67	F	33	153.4	49	6	-	+	-	ND	-
21	1.5	M	52	100.3	30	4	+	+	-	+	-
22	30	F	71	15	13	2	ND	ND	ND	ND	-
23	4	F	96	2.5	94	3	ND	ND	ND	ND	-
24	18	F	99	2.8	40	3	ND	ND	ND	ND	-

For monoclonal antibodies: +, percentage of events more than 20%; -, percentage of events less than 20%; ND, Not done; F, Female; M, Male; BIL, Bilineage acute leukaemia; FAB, French-American-British. **For RT-PCR: +,** positive for *AML1 /ETO* fusion transcript; -, negative for *AML1 /ETO* fusion transcript.

using Wright stain and cytochemical stain according to FAB Cooperative criteria (Bennet *et al.,*1985; 1988; 1976;). Immunophenotyping was performed by indirect staining and FACS analysis, using standard techniques (Bain *et al.,* 2002). Fluorochrome-conjugated McAb for myeloid and lymphoidantigens (Becton Dickinson, BD) were used.

Molecular analysis. Total cellular RNA was extracted from the buffy coat by using single step RNA/DNA isolation kit (Maxim Biotech, Inc) according to the manufacturer's recommendation. A one step reverse transcriptase-polymerase chain reaction (RT-PCR) technique was carried out using the Titan TM One Tube RT-PCR kit (Roche). A volume of 5 ì l of RNA template was diluted in the total of 50 ì l PCR mixture containing 0.2mM dNTPs, 5mM DTT solution, 5U RNase inhibitor, RT-PCR reaction buffer with 7.5 mM $MgCl_2$ and DMSO and a mixture of enzymes. A set of forward primers (5'-TACCACAGAGCCATCAAA-3') located in *AML 1* gene and reverse primers (3'-GTTGTCGGTG-TAAATGAA A-5') located in the *ETO* gene (Downing *et al.,* 1993) were used with a final concentration of 0.4 ì M for PCR. After incubation at 50°C for 30 minutes, the DNA amplification was carried out in a thermocycler for 37 cycles with the following parameters; one denaturation cycle at 94°C for 2 minutes and 10 cycles at 94°C for 30 seconds, 56°C for 30 seconds and 68°C for 45 seconds followed by 25 cycles at 94°C for 30 seconds, 56°C for 30 seconds and 68°C for 45 seconds. Finally, one cycle at 68°C for 45 seconds was performed. The PCR product was subjected to electrophoresis on a 1.5 % agarose gel. The expected size of the PCR product was 185 bp (Downing *et al.,* 1993). A known sample positive for t(8; 21) was used as a positive control.

RESULTS

Twenty-four patients diagnosed as AML in Hospital Universiti Sains Malaysia were analyzed. There were 21 Malays, 2 Chinese, and 1 Siamese. Their races were assigned based on family names. The median age was 24 years(ranging from 2 to 67

years). There were 12 males and 12 females (M:F ; 1:1). There were 10 patients with age less than 15 years. The median and range for haemoglobin, total white blood cells and platelets were 75 gm/L (23 – 118 gm/L), 22.1 x 10^9/l (0.8 – 393.5 x 10^9/l) and 33.5 x 10^9/l (5 – 170 x 10^9/l) respectively. The patients' clinical data are presented in Table 1. Eight patients were classified as AML-M2, five AML-M3, two AML-M1, three AML-M4, one AML-M6 and two AML-M7 according to the FAB classification (Bennet *et al.*, 1985). Three were classified as bilineage acute leukaemia. Seventeen patients had their immunophenotyping done and out of these 5 had CD19 co-expression. Of these 5 patients, 3 were classified as AML-M2 and the other two had additional lymphoid markers and were classified as acute bilineage leukaemia according to accepted scoring system. One of the patients had t(8; 21) by karyotypic analysis and was used as the positive control.

AML1/ETO transcript was detected in 4 (16.7%) out of 24 patients studied where 2 were detected in children aged 10 and 13 years. Based on morphology the transcript was detected in those with AML-M2 (3/24) and AML-1(1/24). Three out of 4 patients positive for the transcript expressed both lymphoid marker (CD19) and the myeloid markers (CD13, CD33).

DISCUSSION

AML1/ETO is a fusion transcript resulting from the translocation of chromosome 8 and chromosome 21. The increased interest in the detection of *AML1/ETO* is its association with relatively good prognosis with remission rates of 80% and disease free survival of 60% (Langabeer *et al.*,1997; Ferrara *et al.*, 2002). In the new WHO classification, AML is classified into four different subgroups (Chillon *et al.*, 2001). AML with recurrent cytogenetic translocation, AML with multilineage dysplasia, AML and myelodysplastic syndrome (MDS) related to previous therapy, and AML not otherwise characterized. *AML 1/ETO* is one of the four different characterized types of translocation in the first group.

The aim of this study was to detect *AML1/ETO* fusion transcript in patients admitted to our hospital and to investigate its relation with the morphological and immunological phenotype.

Though the sample size was small in this study, AML-M2 was the most common morphological subtype with the frequency of 33.3% (8 out of 24 cases) followed by AML-M3, which is 20.7% (5 out of 24 cases). This finding is similar to previous study in Japanese patients where AML-M2 had the highest frequency (29.2%) followed by AML-M3 (21.3%) (Nakase *et al.*, 2000). However in another study in an Australian population, it was found that AML-M4 was 35.6% and AML-M2 was 23.3% (Nakase *et al.,* 2000). In this study AML-M1 was not common where out of 24 cases only two (8.2%) were diagnosed to have AML-M1.

Using a single pair of primers for RT-PCR, *AML1/ETO* fusion transcript was detected in 4 out of 24 patients diagnosed as AML. One sample was sent to an advanced center for cytogenetic analysis and the results showed the presence of t(8;21). This sample also showed fusion transcript on molecular analysis. *AML1/ETO* fusion transcript was detected in 16.67% of cases diagnosed as AML in this study as compared to data from European countries where this aberration is found in only 8% of AML (Nakase *et al.*,2000).

Thus this study showed a higher frequency of *AML1/ETO* fusion transcript in AML-M2 patients compared to other subtypes with the frequency of 12.5% (3 out of 24 patients). Previous studies have suggested a higher frequency of t(8;21) abnormality in AML-M2 in Asian populations, ranging from 55-88%, 19-54% in Europe and 12-27% in USA.

Other than AML-M2, this fusion transcript is also found in AML-M1, and rarely in AML-M4 and other myeloproliferative disorders (de Greef and Hagemeijer,1996; Andrieu *et al.*,1996). It was observed that one of the AML-M1 patients had *AML1/ETO* transcript with an incidence of 4.2% (1 out of 24 patients) and its significance is uncertain because of limited number of samples.

Regarding the age of the patients presenting with AML, it is interesting to note that most of them with the transcript were less than 34 years of age and two of them less than 15 years old, a similar finding noted by Andrieu *et al.*, 1996. From a study reported by UK Medical Research Council, there were 1,065 patients with median age of 66 years and t(8;21) was found in only 23 patients (2.1%). Such a low incidence makes it difficult to evaluate the prognostic impact of t(8;21) in elderly individuals with AML (Langabeer *et al.,*1997).

Immunological analysis on seventeen patients revealed that CD19 was expressed in 5 patients in whom 3 were AML-M2 with *AML1/ETO* fusion transcript (Ferrara and Del Vecchio, 2002; Andrieu *et al.*, 1996). The other two were classified as bilineage with other lymphoid markers expression. HLA-DR and CD33 were also strongly expressed in patients with this transcript.

In conclusion, there was a high frequency of AML-M2 subtype in the population studied. The *AML1/ETO* fusion transcript was detected in 16.7% of AML comparable with other reports (Wong *et al.*, 1993; Maruyama *et al.,*1993; Andrieu *et al.,*1996). It was detected frequently in AML-M2 with a CD19 co-expression. Technically *AML1/ETO* gene could be efficiently identified by one step RT-PCR (Roche) and this technique is feasible to be conducted in future studies for the detection of *AML1/ETO* in leukaemia patients in this institution. It is also a useful method to identify this subgroup of AML in the absence of a readily available cytogenetic service.

ACKNOWLEDGEMENTS

We are grateful to the clinicians from the Medical and Pediatric Department, who have provided materials and to Assoc Prof Dr Normah Jamaludin, Head of Hematology Department for her support. We thank Cik Selamah Ghazali for her technical assistance. This work was supported by USM short-term grant (331/0500/3107).

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