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SHORT REPORT

Incidence and prognosis of c-KIT and FLT3 mutations in core binding factor (CBF) acute myeloid leukaemias

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Summary. DNA from 110 adult *de novo* acute myeloid leukaemia (AML) patients exhibiting either inv(16) ($n = 63$) or t(8;21) ($n = 47$) was screened for mutations in the c-*KIT* (exon 8 and Asp816) and *FLT3* (ITD and Asp835) genes. c-*KIT* exon 8 mutations were found in 15/63 (23·8%) inv(16) patients and 1/47 (2·1%) t(8;21) patients. c-*KIT* Asp816 mutations were present in 5/63 (7·9%) inv(16) AML and 5/47 (10·6%) t(8;21) AML. *FLT3* mutations were identified in five patients (7·9%) with inv(16) and three patients (5·6%) with t(8;21) AML. All mutations were mutually exclusive; 40% of inv(16) AML patients possessed either a c-*KIT* or *FLT3* mutation. c-*KIT* exon 8 mutations were shown to be a significant factor adversely affecting relapse rate.

Keywords: c-KIT, FLT3, AML, CBF, inv(16).

The core binding factor (CBF) leukaemias, inv(16)(p13q22) and t(8;21)(q22;q22), express the fusion genes *CBFβ/MYHII* and *AML1/ETO* respectively. The mechanism by which *AML1* and *CBFβ* disruption induces leukaemogenesis is unclear, although fusion gene expression is not sufficient to cause leukaemia. For example, transgenic mice expressing *AML1/ETO* do not develop leukaemia, unless treated with the mutagenic agent N-ethyl-N-nitrosourea (ENU) (Yuan *et al*, 2001). Similarly, mice transgenic for the chimaeric gene *CBFβ/MYH11* exhibit a myeloid maturation block but require additional mutations for the development of leukaemia (Castilla *et al*, 1999). This suggests that *AML1/ETO* and *CBFβ/MYH11* may dictate the disease phenotype, but that additional 'hits' are required for leukaemic transformation.

Recently, Gilliland (2001) proposed that acute myeloid leukaemia (AML) results from two classes of mutation, a class I mutation that confers a proliferative signal [e.g. a RTK (receptor tyrosine kinase) or RAS mutation], and a class II mutation that impairs haematopoietic differentiation, such as the CBF fusion genes. RTK class III mutations have been linked to a number of haematological malignancies. For example, *FLT3* internal tandem duplication (ITD) and Asp 835 mutations occur in 30% of AML patients and confer a poor prognosis, while *c-KIT* Asp816 mutations are associated with mast cell disease (see Reilly, 2002). In support of the 'two-hit' hypothesis, *FLT3* Asp835 and *c-KIT* Asp816 activating loop mutations have been reported in patients with CBF AML (Beghini *et al*, 2000; Kottaridis *et al*, 2001), while a strong association between *c-KIT* exon 8 mutations and inv(16) AML has been reported (Gari *et al*, 1999).

We report that 40% of patients with AML and inv(16) possessed a class I mutation, supporting Gilliland's pathogenetic model, and that *c-KIT* exon 8 mutations are associated with an increased relapse rate in AML and inv(16).

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MATERIALS AND METHODS

Patient DNA samples and mutation detection. RNA and genomic DNA were obtained from the bone marrow or blood of 110 patients with CBF AML; 63 patients with inv(16)(p13q22) and 47 patients with t(8;21) (see Table 1A for clinical details). Patients were treated in the UK Medical Research Council (MRC) AML 10 and 12 trials ($n = 65$) and the Dutch–Belgian Haematology–Oncology Group HOVON Trials ($n = 45$). Median follow-up was 46 months. Amplification of the FLT3 ITD and Asp835 mutations were performed as described by Abu-Duhier *et al* (2000, 2001). *c-KIT* exon 8 mutations were analysed according to Gari *et al* (1999) while *c-KIT* 816 mutations

Table I.
(A) Clinical details of the 110 CBF AML patients studied.

	inv(16)	t(8;21)	Total
Patients (*n*)	63	47	110
Males (*n*)	36	13	68
Females (*n*)	27	16	42
Mean age (years)	43·9	39·6	42
Age range (years)	15–74	16–83	15–83

(B) Results of *c-KIT* and *FLT3* mutational analysis of the 110 CBF AML patients studied.

	inv(16)	t(8;21)	Total
Patients (*n*)	63	47	110
c-KIT 419	23·8% (15)	2·1% (1)	18·8% (16)
c-KIT 816	7·9% (5)	10·6% (5)	9·1% (10)
FLT3 ITD	3·2% (2)	4·2% (2)	3·6% (4)
*FLT3*835	4·8% (3)	2·1% (1)	3·6% (4)
Total percentage	39·7% (25)	19·1% (9)	30·9% (34)

Data are given as the percentage (number of patients).

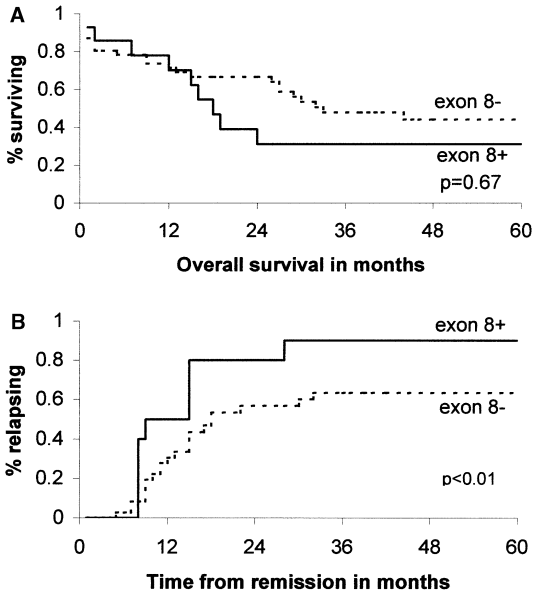

Fig 1. Kaplan–Meier plots showing overall survival (A) and relapse (B) of inv(16) patients with (exon8$^+$) or without (exon8$^-$) an insertion/deletion in c-*KIT* exon 8 involving Asp419.

were analysed by the amplification of exon 17 using 1·1× Readymix PCR master mix containing 1·5 mmol/l $MgCl_2$ (ABgene, Surrey, UK) and 50 nm of each primer: E17F, 5′-TCCATCACCGGTACCTCCTA; and E17R, 5′-CACCACA-GTGAGTGCAGTTG. cDNA, prepared from 50 ng of RNA using random hexamer priming, was also used for mutation detection in some patients.

Statistical analysis. Survival curves were determined by the Kaplan–Meier method (Kaplan & Meier, 1958) and compared as described by Mantel and Haenszel (1959). Overall survival was measured from diagnosis to death or last follow-up, with March 2002 as the final analysis date. Disease-free survival was measured from achievement of complete remission to date of first relapse, death or last follow-up.

RESULTS

Incidence of c-KIT and FLT3 mutations

Of 63 AML patients with inv(16), 23·8% possessed a *c-KIT* exon 8 mutation (Table 1B) that involved Asp419 in 93% of cases. Deletions varied from 6 to 13 bp and insertions from 1 to 15 bp. A single patient, who retained the Asp419 c-*KIT* residue, had only an insertion. Five patients (7·9%) possessed a c-*KIT* Asp816 mutation. *FLT3* ITD and Asp835 mutations were only found in 3·2% and 4·8% of patients respectively. Mutations were mutually exclusive, so that overall 39·7% of patients with AML and inv(16) possessed a RTK mutation. Nine mutations were detected in the 47 patients with t(8;21): c-*KIT* Asp816 ($n = 5$), c-*KIT* exon 8 ($n = 1$), *FLT3* ITD ($n = 2$) and FLT3 Asp835 ($n = 1$) (Table 1B). Overall, 19·1% of patients with t(8;21) possessed a RTK mutation.

Prognostic impact of c-KIT and FLT3 mutations

Overall survival of patients with AML and t(8;21) ($n = 47$) was 61% [95% confidence interval (CI) 51·8–70·1] at 5 years compared with 41% (95% CI 35·7–46·3) for patients with AML and inv(16) ($n = 63$). Kaplan–Meier plots revealed c-*KIT* exon 8 mutations to be a significant factor adversely affecting relapse rate ($P < 0·01$), but not overall survival ($P = 0·67$). (Fig 1A and B).

DISCUSSION

Recently, an intriguing association between c-*KIT* mutations and CBF AML has been documented. Beghini *et al* (1998), for example, reported an Asp816Tyr activating mutation in an AML-M2 with t(8;21), and later strengthened this link by documenting Asp816 mutations in 4/9 patients with t(8;21) and 2/6 patients with inv(16) (Beghini *et al,* 2000). In addition, Gari *et al* (1999) reported *c-KIT* exon 8 mutations with consistent loss of Asp419 in a third of patients with AML-M4Eo and inv(16).

The present study has extended these findings and indicated that 25% of AML and inv(16) patients exhibit a *c-KIT* exon 8 mutation. These data, together with the five additional patients exhibiting an Asp816 mutation, showed that a third of AML patients with inv(16) had a c-*KIT* mutation. *c-KIT* mutations were less common in patients with t(8;21) (12·7%) and were not found in over a hundred patients with CBF-negative AML (results not shown). Although AML patients with inv(16) or t(8;21) generally have a better outcome following chemotherapy, there is still a significant proportion of patients who will relapse following an initial complete remission. From the analysis presented here, it appears that inv(16) AML with *c-KIT*

exon 8 mutations are associated with a greater probability of relapse following complete remission. Thus, the molecular characterization of inv(16) AML, a heterogeneous category of AML in terms of prognosis, may allow the identification of a subset with higher risk disease. Interestingly, RTK mutations appeared to be mutually exclusive, so that 40% of inv(16) patients possessed either a *c-KIT* or *FLT3* mutation. We speculate that the remaining 60% of inv(16) patients should have at least one additional class I mutation.

In conclusion, current evidence suggests that AML results from the collaboration of at least two classes of mutation: class I mutations that confer a proliferative and/or survival advantage and class II mutations that impair differentiation. We believe that our finding of a high frequency of RTK mutations in CBF AML, especially patients expressing *CBFβ/MYH11*, supports this model.

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