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/AML1 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 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).

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 = 63) 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
Overall, 19 KIT patients with t(8;21): c-RTK mutation. Nine mutations were detected in the 47 overall 39 respectively. Mutations were mutually exclusive, so that mutations were only found in 3 (AE 7% of patients with AML and inv(16) possessed 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) 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).

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.
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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REFERENCES


