Double CEBPA mutations, but not single CEBPA mutations, define a subgroup of acute myeloid leukemia with a distinctive gene expression profile that is uniquely associated with a favorable outcome

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Brief report

Double CEBPA mutations, but not single CEBPA mutations, define a subgroup of acute myeloid leukemia with a distinctive gene expression profile that is uniquely associated with a favorable outcome

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Mutations in CCAAT/enhancer binding protein α (CEBPA) are found in 5% to 14% of acute myeloid leukemia (AML) and have been associated with a favorable clinical outcome. Most AMLs with CEBPA mutations simultaneously carry 2 mutations (CEBPA-double-mut), usually biallelic, whereas single heterozygous mutations (CEBPA-single-mut) are less frequently seen. Using denaturing high-performance liquid chromatography and nucleotide sequencing, we identified among a cohort of 598 newly diagnosed AML cases a subset of 41 CEBPA mutant cases (28 CEBPA-double-mut and 13 CEBPA-single-mut cases). CEBPA-double-mut cases associated with a unique gene expression profile as well as favorable overall and event-free survival, retained in multivariable analysis that included cytogenetic risk, FLT3-ITD and NPM1 mutation, white blood cell count, and age. In contrast, CEBPA-single-mut AMLs did not express a discriminative signature and could not be distinguished from wild-type cases as regards clinical outcome. These results demonstrate significant underlying heterogeneity within CEBPA-mutation-positive AML with prognostic relevance. (Blood. 2009;113:3088-3091)

Introduction

Mutations in the transcription factor CCAAT/enhancer binding protein α (CEBPA) are found in 5% to 14% of acute myeloid leukemia (AML).1-9 CEBPA mutations have been associated with a relatively favorable outcome and have therefore gained interest as a prognostic marker.4,6,10 Although variable sequence variations have been described, 2 prototypical classes of mutations are most frequent. N-terminal mutations are located between the major translational start codon and a second ATG in the same open reading frame. These mutations introduce a premature stop of translation of the p42 CEBPA protein while preserving translation of the p30 isoform that has been reported to inhibit the function of full-length protein.7 Mutations in the C-terminal basic leucine zipper (bZIP) region, in contrast, are in-frame and may impair DNA binding and/or homodimerization and heterodimerization.8 The remaining mutations are mostly found between the N-terminus and the bZIP region.11

Most CEBPA mutant AMLs exhibit 2 mutations, which most frequently involves a combination of an N-terminal and a bZIP gene mutation.7,8,11,12 In AMLs with 2 CEBPA mutations, the mutations are typically on different alleles.11 Hence, in these cases, no wild-type CEBPA protein is expressed. A similar condition, whereas single heterozygous mutations and thus retain expression of a wild-type allele.7,11,12

To obtain better insight into the distribution of the various types of CEBPA mutations in de novo adult AML and their impact on clinical outcome, we examined a cohort of 598 cases. After denaturing high-performance liquid chromatography (dHPLC) and nucleotide sequencing, we distinguished cases with 2 different mutations or one homozygous mutation (further referred to as double mutations; CEBPA-double-mut) as well as cases with only one single heterozygous mutation (CEBPA-single-mut). Genome-wide gene expression profiling revealed that CEBPA-double-mut AMLs expressed a highly characteristic signature, whereas CEBPA-single-mut cases did not. In addition, favorable prognosis appeared uniquely associated with CEBPA-double-mut AML.

Methods

AML samples, mRNA isolation, dHPLC analysis, and nucleotide sequencing

Bone marrow aspirates or peripheral blood samples of 598 cases of de novo AML were collected, blast cells were purified, and mRNA was isolated as reported.14 The entire CEBPA coding region was investigated by dHPLC and selected regions also by agarose gel analysis and/or nucleotide sequencing. For details on patient characteristics and experimental procedures, see Document S1 (available on the Blood website; see the Supplemental Materials link at the top of the online article). All studies were approved by the Erasmus University Medical Center Institutional Review Board (Rotterdam, The Netherlands), and patients’ informed consent was obtained in accordance with the Declaration of Helsinki.

Statistical analysis

Survival was estimated according to the method of Kaplan and Meier. The log rank test was used to assess statistical significance. Multivariable analysis was performed using Cox proportional hazards models. Definitions of outcome parameters and cytogenetic risk groups have been described.15 Further details are given in Document S1. P values less than .05 were considered statistically significant.


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Gene expression profiling analysis

Gene expression profiles were obtained using Affymetrix (Santa Clara, CA) HGU133Plus2.0 GeneChips. Details on data processing and analysis are given in Document S1.

Results and discussion

In a cohort of 598 cases of adult de novo AML, we identified 65 cases with an aberrant profile in at least 1 of the 3 investigated amplicons of the CEBPA coding sequence (Figure 1A,B). The presence of a CEBPA sequence variation was confirmed by nucleotide sequencing. Cases that only carried an insertion polymorphism or variation(s) that did not lead to amino acid changes were considered wild-type. Two additional specimens were not considered in further analysis because they carried in-frame variations of unknown significance in the N-terminus (Table S1). As a result, 41 of 598 unambiguous CEBPA\textsuperscript{mut} AML cases (6.9%) were considered. These included 13 CEBPA\textsuperscript{single-mut} cases and 28 CEBPA\textsuperscript{double-mut} cases. Four of the CEBPA\textsuperscript{double-mut} cases carried homozygous mutations, whereas the remaining 24 cases showed 2 heterozygous mutations (Table S1). Additional screening of the remaining AML cases, using a combination of agarose gel analysis and nucleotide sequencing as described,\textsuperscript{8} did not reveal mutations that had been missed by dHPLC.

To investigate whether CEBPA mutations related to gene expression, we examined genome-wide gene expression data of 524 AML cases, which included 26 CEBPA\textsuperscript{double-mut} and 12 CEBPA\textsuperscript{single-mut} cases. Clinical and molecular characteristics of the AML cases are reported in Tables S4 and S5. Using prediction analysis for microarrays,\textsuperscript{18} according to a limited ability to recognize all mutations related to gene expression, we derived a 19-probe set signature for CEBPA\textsuperscript{mut} AML (irrespective of single- or double-mutant status) that was derived in a dataset of 524 AMLs, including 38 CEBPA\textsuperscript{double-mut} cases. Prediction accuracy for each of the 38 CEBPA\textsuperscript{double-mut} cases was estimated using repeated 10-fold cross-validation, as detailed in supplemental data. The proportion of correct predictions for the selected 38 CEBPA\textsuperscript{double-mut} specimens is indicated (top panel). Mutation status is color coded (CEBPA\textsuperscript{single-mut}, blue; CEBPA\textsuperscript{double-mut}, red). The heatmap in the bottom panel depicts the 19 probe sets in the resulting CEBPA\textsuperscript{mut} gene expression classifier (Table S2, probe set information). Intensity values (log2) were mean centered over the cohort of 524 AML cases; and for visualization purposes, the genes were hierarchically clustered (Euclidian distance, average linkage). Cells represent relative log2 expression values and have been color coded on a scale ranging from bright green (−3) to bright red (+3), with black indicating no change relative to the mean. (D) Kaplan-Meier estimates of overall survival among CEBPA\textsuperscript{mut} and CEBPA\textsuperscript{wt} AML (log rank test, P = .027). (E) Overall survival among CEBPA\textsuperscript{single-mut} and CEBPA\textsuperscript{double-mut} AML (P = .008) and versus CEBPA\textsuperscript{mut} AML (P = .005; pooled P = .012). (F) Event-free survival (EFS) among CEBPA\textsuperscript{single-mut} and CEBPA\textsuperscript{double-mut} AML (P = .005) and versus CEBPA\textsuperscript{mut} AML (P = .004; pooled P = .008). The cumulative proportion of survival at the intercept (the point where a line crosses the y-axis) reflects the proportion of patients reaching complete remission. Analyses similar to those depicted in panels D-F were performed after splitting the group of CEBPA\textsuperscript{mut} AMLs into those with favorable cytogenetics and those with other cytogenetics. These additional analyses can be found in Figure S4.
We next assessed how these differences between CEBPΔdouble-mut and CEBPΔsingle-mut related to clinical outcome. In line with previous data, overall survival and event-free survival were significantly better for CEBPΔmut cases compared with cases with wild-type CEBPΔ. (CEBPΔ) (Figure 1D; and data not shown). Separate analyses for the CEBPΔdouble-mut and CEBPΔsingle-mut subgroups, however, revealed a favorable outcome that was specific for CEBPΔdouble-mut cases. We failed to find a favorable prognostic effect in relation to the CEBPΔsingle-mut cases. Indeed, CEBPΔsingle-mut AMLs showed a significantly worse outcome than CEBPΔdouble-mut cases, including a poor rate of complete remission (Figure 1E,F). These findings were also apparent in multivariable analysis (Table 1). When only patients younger than 60 years or only patients with normal cytogenetics were considered, similar results were found, although in the latter subgroup with smaller numbers only the pairwise comparison for overall survival between CEBPΔdouble-mut and CEBPΔsingle-mut reached statistical significance (Figure S3; Table S6).

Based on our previous analyses6 and on the literature,11 it is probable that, in the majority of the CEBPΔdouble-mut AML studied, both CEBPΔ alleles were affected. A plausible hypothesis is therefore that absence of wild-type CEBPΔ mRNA is directly involved in the CEBPΔdouble-mut gene expression profile. This may be further supported by our previous and current observations that indicate a high degree of similarity between the profiles of CEBPΔdouble-mut AML and a specific subgroup of leukemias characterized by epigenetic CEBPΔ silencing (Figure S1).19 It is possible that analysis of larger patient series will lead to further refinement of this subclassification, for instance, based on the location of the mutations. For example, our data indicated a tendency of CEBPΔsingle-mut cases with mutations in the bZIP region to be potentially less distinct from the CEBPΔdouble-mut AMLs (case nos. 7185, 7324, and 2237; Figures 1C, S2). Of note, a subset of the CEBPΔmut AMLs studied here was included in the cohort of 285 cases of AML that we previously investigated by gene expression profiling.14 In that study, all CEBPΔdouble-mut AMLs were found in 2 particular clusters, whereas CEBPΔsingle-mut AMLs did not specifically aggregate.14,19

Studies to date have associated CEBPΔ mutations with outcome6,8,9 but have not applied subdivisions into single and double mutants. It is unclear why CEBPΔdouble-mut AMLs would have a better outcome than those with a single heterozygous mutation. One explanation could be that a single-mutant CEBPΔ allele is not sufficient for leukemogenesis and requires cooperating mutations, which may be in CEBPΔ itself or in other genes. Of note, recent data indicate that germline CEBPΔ mutations predispose to AML, and the acquisition of a second, somatic CEBPΔ mutation may then contribute to AML development.20 Indeed, we found a tendency toward more FLT3-ITD, FLT3-TKD, and NPM1 mutations in CEBPΔsingle-mut compared with CEBPΔdouble-mut cases (Table S5). Yet unknown abnormalities may associate with CEBPΔsingle-mut AML as well and predispose to inferior outcome. It is however evident that these findings and their clinical significance warrant confirmation in independent cohorts of AML.

In conclusion, the data presented here indicate that CEBPΔmut AML should at least be distinguished according to the presence of CEBPΔdouble-mut and CEBPΔsingle-mut. Screening using dHPLC, followed by nucleotide sequencing, appears useful for rapidly identifying mutant cases. In addition, gene expression-based classification, for instance, using the classifiers described here, enables the accurate identification of CEBPΔdouble-mut AML cases.

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Authorship


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University Medical Center (Erasmus MC), held in a Special Purpose Foundation of Erasmus MC. The remaining authors declare no competing financial interests.

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