CARACTERIZACIÓN BIOLÓGICA DE LA LEUCEMIA MIELOIDE AGUDA CON TRANSLOCACIÓN t(8;16)(p11;p13) Y REORDENAMIENTO *MYST3-CREBBP*

Tesi presentada per

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per aspirar al grau de Doctora en Medicina

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III. RESULTADOS

1. PRIMER TRABAJO

Type I *MOZ/CBP (MYST3/CREBBP)* is the Most Common Chimeric Transcript in Acute Myeloid Leukemia with t(8;16)(p11;p13) Translocation.

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Resumen

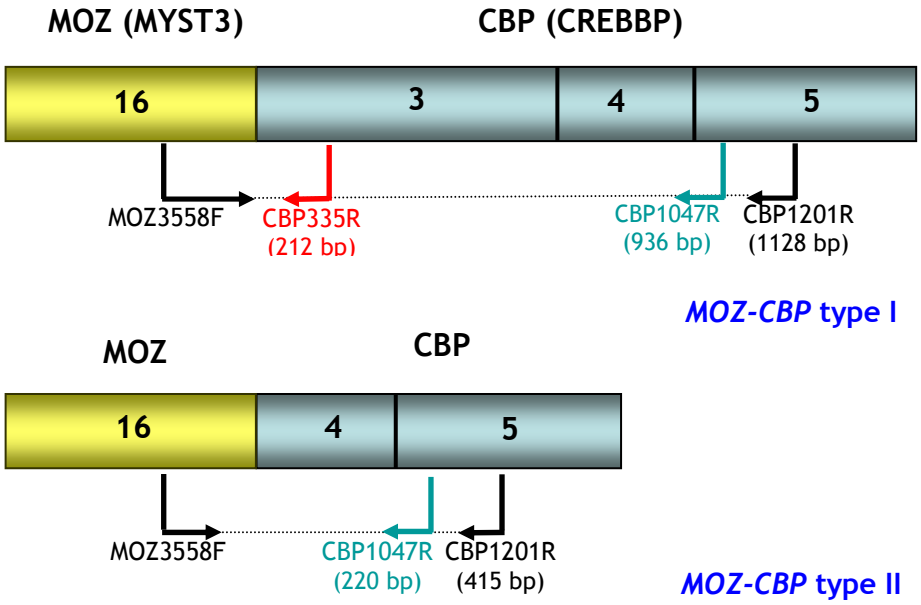
La LMA con t(8;16)(p11;p13) es un tipo de leucemia infrecuente que presenta una serie de características clínico-biológicas comunes, como son una frecuente afectación extramedular, una coagulopatía severa y un mal pronóstico. Los blastos tienen una diferenciación mielomonocítica y presentan de forma peculiar una positividad simultánea a las tinciones citoquímicas de la mieloperoxidasa y esterasas inespecíficas.

A nivel molecular, la translocación da lugar a la fusión del gen *MYST3* (*MOZ),* en 8p11, con *CREBBP* (*CBP*), en 16p13. Hasta el momento del estudio, la amplificación del gen quimérico *MYST3-CREBBP* había sido técnicamente difícil, de forma que tan solo se había descrito en la literatura el estudio mediante RT-PCR de 4 casos, en los que se demostraron 4 tránscritos diferentes, con los puntos de ruptura situados en el intrón 2 del gen *CREBBP* y en el intrón 16 (tránscrito tipo I) o el exón 17 del gen *MYST3*.

En este trabajo se estudiaron las características morfológicas, inmunofenotípicas y moleculares de una serie de pacientes afectos de LMA, con la t(8;16)(p11;p13) confirmada citogenéticamente (n=5) o bien con características morfológicas o citoquímicas similares, que aconsejaban descartar la presencia del reordenamiento *MYST3-CREBBP* (n=13).

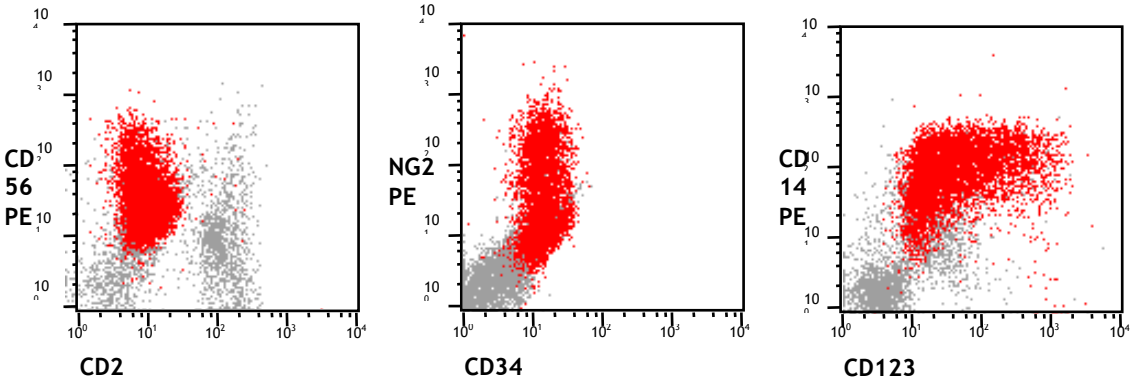
Para la detección molecular del reordenamiento *MYST3-CREBBP* inicialmente utilizamos una PCR semi-anidada. De esta manera, para la primera PCR se siguió la técnica descrita en la literatura, utilizando los oligonucleótidos MOZ3558F y CBP1201R. El producto resultante fue de nuevo sometido a amplificación con los oligonucleótidos MOZ3558F y CBP1047R, un nuevo cebador más interno localizado en el exón 5 de *CREBBP*. Posteriormente diseñamos una nueva RT-PCR con el objetivo de amplificar el denominado tránscrito tipo I, utilizando el oligonucleótido MOZ3558F ya mencionado combinado con un nuevo oligonucleótido, CBP335R, situado en el exón 3 de *CREBBP*. Con esta estrategia se pretendía amplificar un fragmento de tan sólo 212 pb (ver figura 18 y figura 2a del primer trabajo).

Figura 18. Localización de los oligonucleótidos utilizados en el trabajo. En color negro se detallan los cebadores descritos en la literatura (Panagopoulos et al, 2000); en color verde y rojo se detallan los diseñados en este trabajo para una PCR semi-anidada y una PCR sencilla, respectivamente. En paréntesis sigue a cada cebador el tamaño del producto de c□NA a amplificar.



Con las técnicas citogenéticas y moleculares reunimos una serie de 7 pacientes con LMA y reordenamiento *MYST3-CREBBP* (5 casos detectados por citogenética convencional y dos casos adicionales sin crecimiento de metafases con la presencia del reordenamiento confirmado por métodos moleculares). Las características clínicas y morfo-citoquímicas de nuestros pacientes no fueron diferentes de las descritas en la literatura. El análisis inmunofenotípico puso de manifiesto un perfil homogéneo, con expresión del antígeno HLA-DR, negatividad para CD34 y CD117 y un patrón de diferenciación mielomonocítica, junto con la frecuente expresión aberrante de CD56 y NG2 (figura 19).

Figura 19. Análisis por citometría de flujo de los casos de LMA con t(8;16)(p11;p13): se observó negatividad para C□34, una expresión de antígenos de diferenciación monocítica (C□14) y frecuente expresión aberrante de C□56 y NG2.



Por otro lado, en el estudio molecular mediante la nueva RT-PCR diseñada se amplificó una banda de 212 pb en 6 casos, 4 de ellos con la t(8;16) confirmada por citogenética y dos casos sin estudio citogenético valorable, por la presencia de necrosis medular y por ausencia de metafases, respectivamente. En un paciente con la t(8;16) confirmada por citogenética no se obtuvo producto de amplificación en la PCR, probablemente por degradación parcial del ARN, extraído de parafina. El análisis de la secuencia de los productos amplificados confirmó la presencia del tránscrito tipo I del gen quimérico *MYST3-CREBBP* en todos los casos, con unos puntos de ruptura localizados en el nt 3745 del gen *MYST3* y en el nt 283 de *CREBBP* (ver figura 2b y 2c del trabajo).

Por tanto, de este trabajo, que analiza la serie más larga descrita de casos de LMA con reordenamiento *MYST3-CREBBP* desde el punto de vista molecular, podemos concluir que estos pacientes poseen un inmunofenotipo característico (CD34-, HLA-DR-, CD117-, CD56+, expresión de marcadores mielomonocíticos) y que el tránscrito tipo I del gen quimérico *MYST3-CREBBP* es el más común en estos pacientes. Por otro lado, la técnica de RT-PCR implementada es útil como confirmación del reordenamiento en los casos con t(8;16)(p11;p13) detectada por citogenética y además facilita la detección rápida del reordenamiento *MYST3-CREBBP* en casos sospechosos en los cuales no se ha obtenido una citogenética convencional valorable.

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BRIEF COMMUNICATION

Type I *MOZ/CBP (MYST3/CREBBP)* Is the Most Common Chimeric Transcript in Acute Myeloid Leukemia with t(8;16)(p11;p13) Translocation

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The t(8;16)(p11;p13) fuses the *MOZ* (*MYST3*) gene at 8p11 with *CBP* (*CREBBP*) at 16p13 and is associated with an infrequent but well-defined type of acute myeloid leukemia (AML) that has unique morphocytochemical findings (monocytoid blast morphology with erythrophagocytosis and simultaneously positive for myeloperoxidase and nonspecific esterases). RT-PCR amplification of *MOZ/CBP* (*MYST3/CREBBP*) chimera has proved difficult, with four different transcripts found in four reported cases. We studied 7 AML-t(8;16) patients, 5 with cytogenetically demonstrated t(8;16) and 2 with similar morphocytochemical and immunophenotypical characteristics. Clinically, 3 cases presented as therapy-related leukemia. Extramedullar involvement was observed at presentation in 2 patients and coagulopathy in 4. The clinicobiological findings confirmed the distinctiveness of this entity. Of note is the erythrophagocytosis in 5 of 7 cases and the immunological negativity for CD34 and CD117 and positivity for CD56. Using a new RT-PCR strategy, we were able to amplify a specific band of 212 bp in six cases in which sequence analysis confirmed the presence of the previously described *MOZ/CBP* fusion transcript type I. This is the largest molecularly studied AML-t(8;16) series, which demonstrates that *MOZ/CBP* breakpoints are usually clustered in intron 16 of *MOZ* and intron 2 of *CBP*. The newly designed single-round PCR provides a simple tool for the molecular confirmation of *MOZ/CBP* rearrangement.

Recurrent chromosomal translocations resulting in expression of fusion gene products are frequently observed in acute myeloid leukemia (AML). Most of these cytogenetic abnormalities characterize disease entities with specific clinical and biological features. AML with t(8;16)(p11;p13) [AML-t(8;16)] is an infrequent type of leukemia reported in approximately 50 de novo AML and therapy-related AML (t-AML) cases with distinct clinical and hematological characteristics (Sun and Wu, 2001). AML-t(8;16) patients have frequent extramedullar involvement and coagulation disorders at diagnosis. The prognosis is usually extremely poor, with a median survival of only two months (Hanslip et al., 1992; Stark et al., 1995; Velloso et al., 1996; Sun and Wu, 2001). The proliferating cells are of myelomonocytic lineage, exhibit prominent erythrophagocytosis, and show dual myeloperoxidase (MPO) and nonspecific esterase cytochemical staining. At the molecular level, the t(8;16) translocation fuses *MOZ* (*MYST* histone acetyltransferase–monocytic leukemia–3) gene, located at 8p11, with *CBP* (CREB-binding protein), at 16p13 (Borrow et al., 1996; Aguiar et al., 1997). Although genomic rearrangements of the *MOZ* and *CBP* genes have been identified by fluorescence in situ hybridization and Southern blot (Borrow et al., 1996; Giles et al., 1997), amplification of the *MOZ/CBP* transcript and its reverse, the *CBP/MOZ* transcript, by RT-PCR has proved difficult (Giles et al., 1997; Bernasconi et al., 2000). Thus, only 4 AML-t(8;16) cases analyzed by RT-PCR have been published so far, with recognition of four different *MOZ/CBP* fusion transcripts and

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TABLE 1. Main Clinical and Hematological Characteristics of Patients with AML-t(8;16)

Patient	1	2	3[a]	4	5	6	7
Age/gender	28/M	51/F	19/M	51/F	53/F	79/M	30/F
Onset	De novo	De novo	De novo	Therapy-related	Therapy-related	Previous MDS	Therapy-related
Extramedullar	No	No	Skin and lymph nodes	No	No	Skin, liver, and spleen	No
WBC (10^9/L)	8	40	14	21	12	16	6
DIC	No	No	No	Yes	Yes	No	Yes
BM blasts (%)	92	89	70	NA[b]	96	63	56
Hemophagocytosis	No	Yes	Yes	NA[b]	Yes	Yes	Yes
MPO/NSE	+/+	+/+	+/+	+/+	+/+	+/+	−/+
CD34	−	−	−	−	−	±	−
CD117	−	−	NA	−	−	±	−
HLA-DR	−	+	±	+	+	+	±
CD13	+	+	NA	+	−	+	+
CD33	+	+	NA	+	+	+	+
CD15	+	+	+	+	+	+	+
i-MPO	+	NA	+	+	+	+	+
CD4	+	+	+	+	+	+	±
CD11b	−	+	NA	+	±	+	+
CD11c	+	NA	NA	+	NA	NA	+
CD56	+	+	±	±	−	−	+
Karyotype	46,XY,t(8;16) (p11;p13) [20]	46,XX,t(8;16) (p11;p13) [8]	46,XY,t(8;16) (p11;p13)[20]	NA[b]	46,XX,t(8;16) (p11;p13) [20]	NA	46,XX,t(8;16) (p11;p13) [3]
MOZ/CBP type 1	+	+	NA	+	+	+	+
Outcome	CCR (34+ mos)	CR but relapse at +13 mos	CCR (27+ mos)	Early death (alveolar hemorrhage)	Early death (GI bleeding)	Early death (cerebral hemorrhage)	CR Relapse at +4 and 2nd CR, Dead at +20 (alloSCT)

MDS, myelodysplastic syndrome; DIC, disseminated intravascular coagulation; NA, not assessable.

[a]Phenotyped by immunohistochemistry.

[b]Bone marrow necrosis.

MPO, cytochemical myeloperoxidase; NSE, nonspecific esterases; i-MPO, immunological myeloperoxidase; CCR, continuous complete remission; CR, complete remission; alloSCT, allogeneic stem cell transplantation; Mos, months; GI, gastrointestinal.

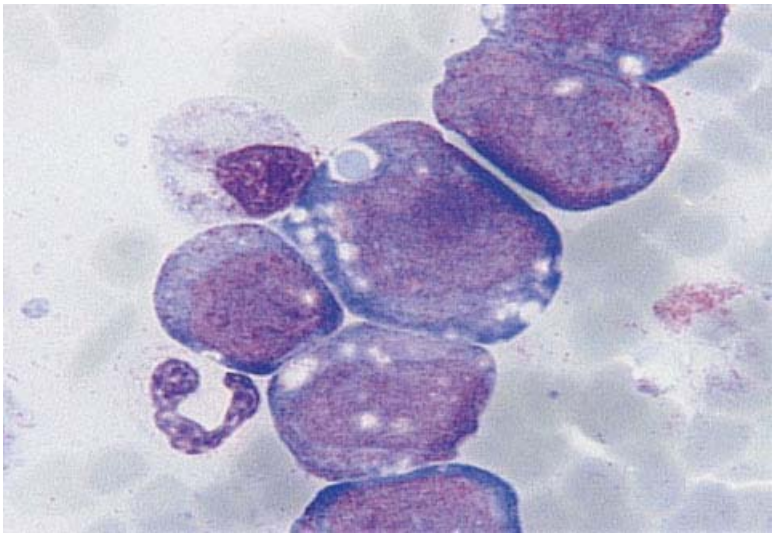

Figure 1. Large blasts with monocytic appearance, heavy granulation, and erythrophagocytosis in a patient with AML-t(8;16). Bone marrow, May-Grünwald Giemsa.

one *CBP/MOZ* isoform (Borrow et al., 1996; Panagopoulos et al., 2000, 2002).

We have identified 7 patients with AML-t(8;16), 5 showing a t(8;16) and the other 2 having similar morphocytochemical and immunophenotypical characteristics but in which the cytogenetic study had failed. These cases were screened for *MOZ/CBP*, together with 11 FAB M4/M5 acute myeloid leukemias. The clinical, immunophenotypical, and cytogenetic findings were collected from the medical records, and the morphological characteristics of the bone marrow, peripheral blood, and tissues were reviewed.

For the study of *MOZ/CBP* rearrangement, we performed RT-PCR using RNA extracted from peripheral blood and/or bone marrow samples in 17 cases and from a lymph node in 1 case. Total RNA was isolated by a modified one-step guanidium thiocyanate-phenol-chloroform method using Ultraspec RNA (Biotecx Laboratories, Houston, TX) as previously reported (Chomczynski and Sacchi, 1987). In 1 of the 5 cases with cytogenetically demonstrated t(8;16), the RNA obtained was not of good quality for RT-PCR analysis. One microgram of total RNA was denatured at 65°C for 5 min, and then reverse transcription was performed with 0.75 U/μL Moloney–murine leukemia virus reverse transcriptase (Invitrogen, Gaithersburg, MD) in the manufacturer's buffer with 0.75 U/μL of RNase inhibitor (Promega, Madison, WI) and 2.5 mM random hexamer primers at 37°C for 1 hr in a final volume of 40 μL. First-round PCR was done in a total volume of 25 μL containing 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μL of Expand High Fidelity *Taq* polymerase (Roche, Mannheim, Germany), 0.25 μM of each of the primers MOZ3558F (5′-GAGGCCAATGCCAAGATTAGAAC-3′) and CBP1201R (5′-GTACCCACACAAGCAATTGCAAC-3′), and 5 μL of the cDNA. After an initial denaturation at 95°C for 5 min, 40 cycles of 1 min at 95°C, 1 min at 60°C, and 1 min at 72°C were run, followed by a final extension for 10 min at 72°C. For the second-round PCR, 2.5 μL of the first PCR product was reamplified using primers MOZ3558F and CBP1047R (5′-AGCTTGACTAAAGGGCTGTC-3′) for the inner reaction. Using these primers, a product of 936 bp corresponding to *MOZ/CBP* transcript type I, and a band of 220 bp corresponding to *MOZ/CBP* transcript type II (accession numbers HSA251843 and HSA251844, respectively), should be amplified. Subsequently, we designed a single-round PCR for amplification of transcript type I using a new reverse primer, CBP335R (5′-GGTATCAGCTCATCAGGAAGATCA-3′) and an annealing temperature of 55°.

For the detection of reciprocal transcript *CBP/MOZ,* we performed nested PCR using the primers CBP96F, MOZ3953R, CBP174F, and MOZ3844R, as previously described (Panagopoulos et al., 2000).

Ten microliters of the PCR products was analyzed by electrophoresis through 2% agarose gels, stained with ethidium bromide, and visualized under UV. The PCR products were purified by gel excision with the QIAEX II agarose-gel extraction kit (Qiagen, Hilden, Germany) and directly sequenced from both strands, using the Big Dye Terminator Cycle Sequencing Ready Reaction (versions 3 and 3.1, Applied Biosystems, Foster City, CA) following the manufacturer's instructions. Sequencing analysis and alignments were

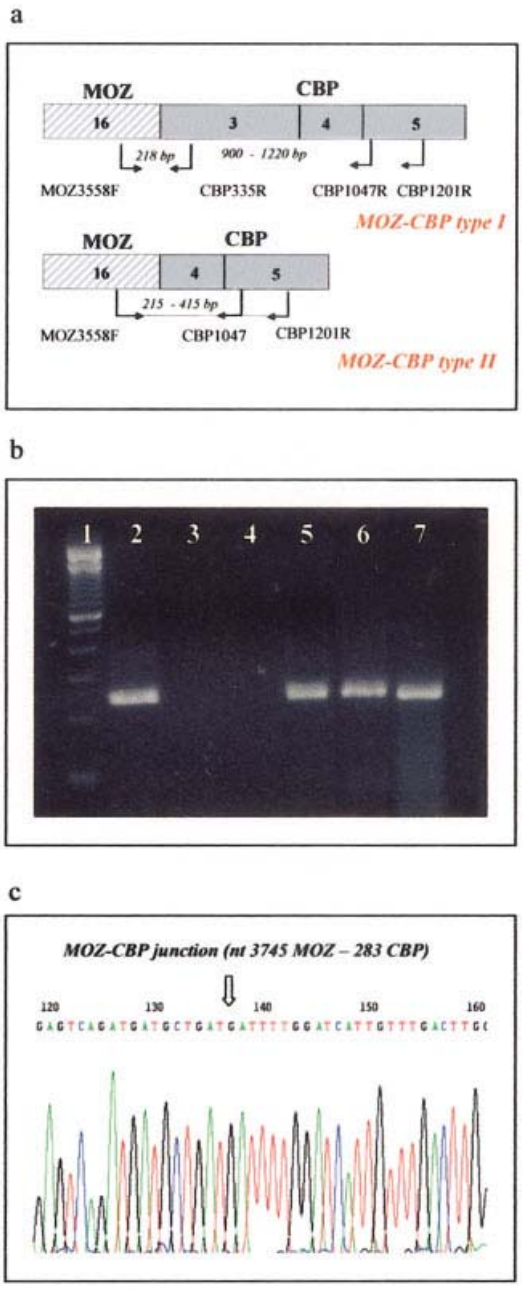

Figure 2. a. Schematic representation of types I and II *MOZ/CBP* chimeric transcripts (accession numbers AJ251843 and AJ251844). The positions of the primers used in the RT-PCR are indicated (not drawn to scale; adapted from Panagopoulos et al., 2000). b. Chimeric *MOZ/CBP* transcripts in four AML cases with t(8;16)(p11;p13). RT-PCR amplification of *MOZ/CBP* transcript type I. Lane 1: 100-bp molecular-weight DNA ladder; lanes 2, 5, 6, and 7: positive AML-t(8;16) samples; lane 3: no cDNA template; and lane 4: negative AML sample. c. Partial sequence chromatogram of the 212-bp amplified fragment, corresponding to mRNA of type I *MOZ/CBP* chimeric transcript, from one AML-t(8;16) representative case.

performed using BLAST software (www.ncbi.nlm.nih.gov/BLAST/).

The main clinical and biological characteristics of the patients are summarized in Table 1. Interestingly, AML arose as t-AML in 3 patients who had received topoisomerase-II inhibitors for a previous neoplasia, and 1 patient had previously had myelodysplastic syndrome. Although an early death from a hemorrhagic event in the context of a severe coagulation disorder was observed in 3 patients, a complete remission (CR) was achieved in 4 cases, 2 of them remaining in durable response after undergoing an allogeneic stem cell transplantation (alloSCT) in the first CR. Therefore, intensive therapy, probably including alloSCT, might cure a proportion of patients with this high-risk AML.

The blasts showed a monocytic appearance with heavy granulation, with erythrophagocytosis in 5 of the 7 and dual MPO/NSE staining in 6 of the 7 cases (Table 1, Fig. 1). Immunophenotyping by flow cytometry or immunohistochemistry disclosed a homogenous profile, with the expression of HLA-DR, the absence of CD34 and CD117, and a myelomonocytic differentiation pattern, in accord with the results in the few published reports (Stark et al., 1995; Sun and Wu, 2001). Of note, CD56 was positive in 5 of 7 cases. Although this feature is related to extramedullar involvement (Baer et al., 1997), only 1 of our 5 CD56+ patients had blastic infiltration of the skin and lymph nodes. This clinicobiological profile may be highly suggestive of AML-t(8;16), but some of their characteristics, such as coagulopathy, heavy granulation of blasts, strong positivity for MPO, and negativity for CD34, may be present in other AML subtypes such as acute promyelocytic leukemia (Sun and Wu, 2001). In this sense, the molecular studies can contribute to the differential diagnosis. Conventional cytogenetics disclosed the t(8;16)(p11;p13) in 5 cases; in the other 2 patients, an informative karyotype was not available because of bone marrow necrosis and the absence of assessable metaphase cells.

To the best of our knowledge, only 4 cases of AML with *MOZ/CBP* rearrangement analyzed by RT-PCR have been reported (Borrow et al., 1996; Panagopoulos et al., 2000, 2002). Panagopoulos et al. (2000) detected two types of *MOZ/CBP* fusion transcripts (types I and II), of 1,128 and 415 bp, respectively, in 2 patients. The sequencing of these fragments disclosed the in-frame fusion of nucleotide (nt) 3,745 of *MOZ* (accession number U47742) with nt 283 of *CBP* (NM_004380) in transcript type I, whereas the same locus of *MOZ* was fused out-of-frame with nt 997 of *CBP* in transcript type II (Fig. 2a; Panagopoulos et al., 2000). Recent genomic studies of these cases localized the breakpoint within intron 16 of *MOZ* (Panagopoulos et al.,

2003). Furthermore, two additional cases have been described; these have breakpoints within exon 17 of *MOZ* (Borrow et al., 1996; Panagopoulos et al., 2002). In our series, we initially followed a slightly modified version of a previously published RT-PCR strategy (Panagopoulos et al., 2000), obtaining 2 weak bands, of 936 bp and 220 bp, in only 2 of the studied cases. We subsequently designed a single-round RT-PCR for the amplification of in-frame transcript type I using the same forward MOZ3558F primer and an inner reverse primer (CPB335R). This strategy yielded amplification of a 212-bp band in all cases with the available RNA (Fig. 2b). Direct sequencing of the PCR product confirmed the presence of the type I *MOZ/CBP* fusion rearrangement, with breakpoints at nt 3,745 of *MOZ* and nt 283 of *CBP* (Fig. 2c). This rearrangement was not found in any of the other 11 M4/M5 AML cases tested. Of note, similar breakpoints within intron 16 at *MOZ* have been described in AML with inv(8)(p11q13) and t(8;22)(p11;q13), which juxtapose *MOZ* to *TIF2* (nuclear receptor coactivator 2) and *EP300* (E1A-binding protein p 300), respectively (Carapeti et al., 1998; Liang et al., 1998; Kitabayashi et al., 2001b). It has been suggested that this site of *MOZ* is prone to breakage, which would explain the frequency of t-AML harboring this rearrangement. An alternative explanation is there being a selective advantage to in-frame hybrids generated in this region (Panagopoulos et al., 2003). Nevertheless, as previously mentioned, alternative *MOZ* breakpoints, in exon 17 in two AML-t(8;16) and in intron 15 in one AML-t(8;22), have been reported (Borrow et al., 1996; Panagopoulos et al., 2000; Kitabayashi et al., 2001b).

The *MOZ* gene is composed of 17 exons and contains a *MYST* domain with histone acetyltransferase (HAT) activity. This domain, in exons 9–14, remains intact in all the t(8;16) translocations described to date (Kitabayashi et al., 2001a; Panagopoulos et al., 2003). *MOZ* modulates the transcription of specific target genes by coactivating the *RUNX1* (runt-related transcription factor 1) transcription factor complex (Champagne et al., 2001; Kitabayashi et al., 2001a). Possible leukemogenic mechanisms derived from the *MOZ/CBP* rearrangement are aberrant chromatin acetylation by the mistargeting of specific HAT activity and an inhibition of *RUNX1*-mediated transcription (Champagne et al., 2001; Kitabayashi et al., 2001b; Panagopoulos et al., 2003). On the other hand, the *CBP* gene is believed to coordinate the transcriptional effects of multiple signals from cell surface and nuclear receptors (Aguiar et al., 1997). *CBP* is also fused to other partners such as *MLL* in t-AML with t(11;16)(q23;p13) and *MORF* (*MYST* histone acetyltransferase–monocytic leukemia–4) in t(10;16)(q22;p13), a gene highly homologous to *MOZ* in structure and function that also breaks within intron 16 (Panagopoulos et al., 2001). All the breakpoints reported in *CBP* chimeras are in intron 2 (Borrow et al., 1996; Aguiar et al., 1997; Giles et al., 1997; Rowley et al., 1997; Panagopoulos et al., 2000, 2002, 2003).

The reciprocal *CBP/MOZ* transcript was not amplified in any of our cases. In some reports, the *CBP/MOZ* transcript was either out-of-frame (Borrow et al., 1996) or not expressed (Panagopoulos et al., 2002). Therefore, *MOZ/CBP*, but not the *CBP/MOZ* transcript, is believed to be of importance in the leukemogenic process (Panagopoulos et al., 2002).

In summary, our cases constitute the largest AML-t(8;16) series with *MOZ/CBP* rearrangement analyzed at the molecular level, showing that breakpoints are clustered in intron 16 of *MOZ* and intron 2 of *CBP* in almost all patients. Moreover, we have designed a single-round PCR that can be a simple tool for the molecular confirmation of *MOZ/CBP* rearrangement.

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3.2. SEGUNDO TRABAJO

Gene Expression Profiling of Acute Myeloid Leukemia with Translocation t(8;16)(p11;p13) and MYST3-CREBBP Rearrangement Reveals a Distinctive Signature with a Specific Pattern of HOX Gene Expression

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Resumen

En la leucemia mieloide aguda con t(8;16)(p11;p13) se produce la fusión de dos genes, *MYST3* y *CREBBP*, con capacidad de remodelar la cromatina mediante la acetilación de histonas (actividad HAT). Este hecho ha llevado a hipotetizar que el mecanismo principal de leucemogénesis en estas leucemias consiste en una acetilación aberrante. Sin embargo, no se conocían con exactitud las vías alteradas por la proteína quimérica MYST3-CREBBP. Además, hasta el momento de iniciar la tesis, el estudio del perfil genómico de las LMA con t(8;16) y reordenamiento *MYST3-CREBBP* no había sido abordado.

En este trabajo se analizó el perfil de expresión génica de una serie de pacientes con reordenamiento *MYST3-CREBBP* mediante microarrays de oligonucleótidos y se comparó con el de otros subtipos bien definidos de LMA. Para ello, de nuestra serie seleccionamos 3 casos de pacientes con LMA y reordenamiento *MYST3-CREBBP*, junto con 20 casos adicionales de LMA de diversos subtipos, definidos principalmente por sus alteraciones citogenéticas (t(15;17)(q22;q12), n=3; t(8;21)(q22;q22), n=3; inv(16)/t(16;16), n=3, y t(9;11)(p22;q23), n=1). También se incluyeron en el análisis 2 casos de LMA con displasia multilínea y 8 casos de LMA con diferenciación monocítica sin las alteraciones citogenéticas mencionadas (ver tabla A del material suplementario). Todos los casos fueron analizados mediante los *arrays* de

oligonucleótidos HU133A (Affymetrix), que contienen 22283 sondas correspondientes a unos 13000 genes.

En el análisis no supervisado se observaron dos ramas principales en el dendrograma, que correspondían a los casos de diferenciación mieloide y diferenciación monocítica, respectivamente (ver figura 1A del trabajo). Además, se identificaron un total de 5 *clusters*, formados por las LMA con reordenamiento *MYST3-CREBBP*, las leucemias con genes quiméricos resultado de las t(15;17), t(8;21), inv(16) y un *cluster* que agrupaba las LMA con displasia multilínea. El resto de casos, constituídos por LMA de diferenciación monocítica sin ninguna de las anomalías anteriormente mencionadas, se distribuyeron entre los demás subtipos formando un grupo heterogéneo.

El análisis supervisado se centró en el estudio, por un lado, de aquellos genes con expresión significativamente diferente en las LMA con reordenamiento *MYST3-CREBBP*. Para ello aplicamos diferentes técnicas estadísticas: en primer lugar se utilizó un test de ANOVA para hallar las diferencias en la expresión de genes entre los diferentes subgrupos de leucemias observados en el análisis no supervisado (ver figura 1B del trabajo, tabla D y figura A del material suplementario); además, los datos fueron analizados con la prueba *t* de student para comparar la expresión de genes en la LMA-*MYST3-CREBBP* con el conjunto de los otros tipos de leucemias (tablas G y H del material suplementario). Entre los genes que se encontraron sobreexpresados de manera significativa en el subgrupo de pacientes con reordenamiento *MYST3-CREBBP* (n=63 y n=53 genes con los análisis de ANOVA

y t-test, respectivamente) destacaban algunos oncogenes (*RET, PRL*), genes involucrados en la transcripción (*HOXA10, PPARG*), en daño celular (*IRAK1*), reparación de DNA (□□*B2*), apoptosis (□*AP, OPTN*) y otros, como *C20orf103* y *CEBPA*. Por el contrario, el gen *ciclina* □*2 (CCN*□*2)* y los genes de la familia *RAS RAB6A* y *RAB8A* estaban infraexpresados en estas leucemias.

Por otro lado, se intentó identificar aquellos genes con una expresión similar en las LMA con reordenamiento *MYST3-CREBBP* y alguno de los otros subtipos de leucemia. Así, se observó que las LMA-*MYST3-CREBBP* compartían parcialmente un perfil de expresión de genes con el resto de leucemias de estirpe monocítica y, en menor medida, con alguna de las otras categorías de leucemias (tablas E y F del material suplementario).

En la segunda parte del estudio se seleccionaron 46 genes diferencialmente expresados en las LMA-*MYST3-CREBBP* (ver listado en la tabla C del material suplementario) y se analizó su expresión mediante RT-PCR cuantitativa con arrays de baja densidad en una serie independiente de 40 pacientes con LMA. En este segundo grupo de pacientes estudiados se incluyeron las tres muestras de LMA-*MYST3-CREBBP* ya analizadas con microarrays y 4 muestras adicionales con el mismo reordenamiento pero sin material apropiado para el estudio del PEG. Además, se incluyeron muestras de los principales subtipos de LMA (t(15;17), t(8;21), inv(16)), así como casos de LMA con reordenamiento de *MLL*, ya fuera en forma de translocaciones (t-*MLL*) o de una duplicación parcial en tándem del gen (*MLL*-PTD). Por último, se incluyó un grupo de casos de LMA con diferentes cariotipos y diversos

grados de maduración (ver tabla B del material suplementario). La expresión relativa de los genes amplificados mediante RT-PCR cuantitativa fue analizada con el método ΔΔCt.

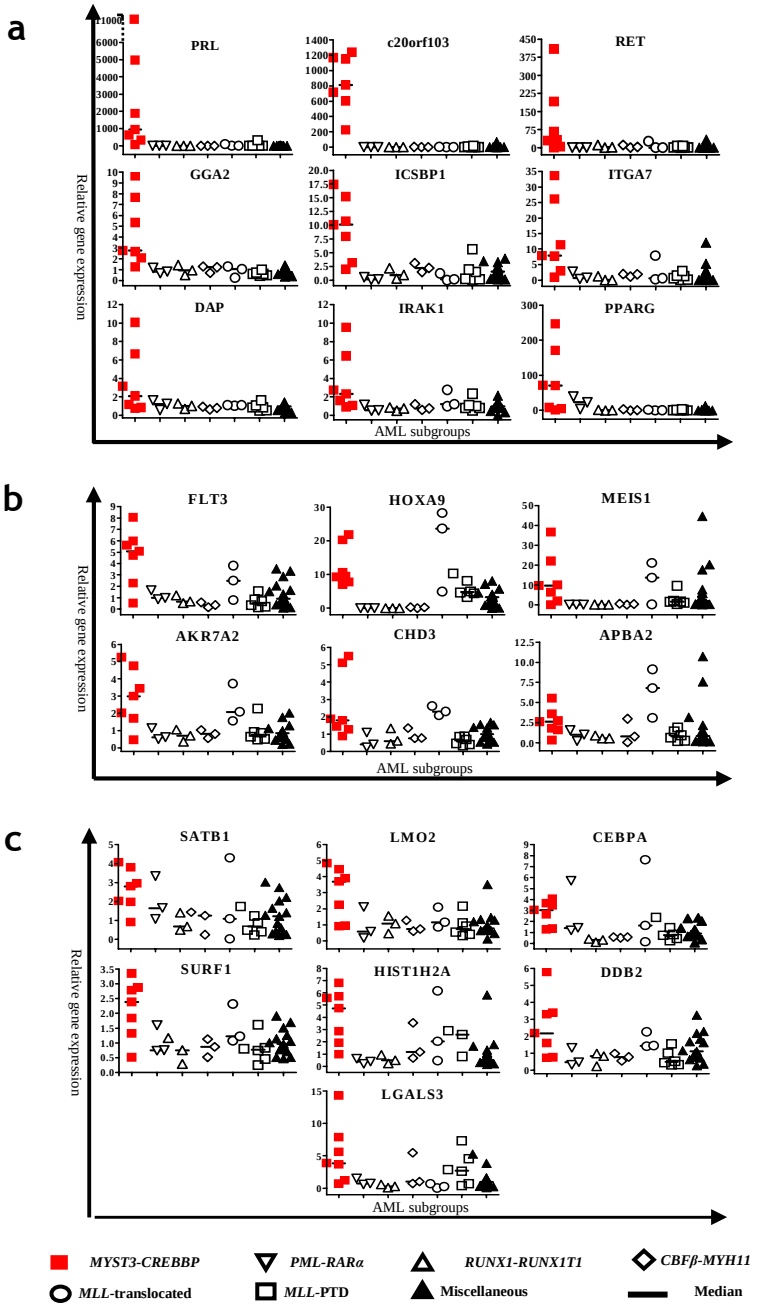
Para definir el grupo de genes característico de las LMA con reordenamiento *MYST3-CREBBP* se utilizó un t-test comparando dicho subtipo con el resto de leucemias. Con este análisis se observaron 22 genes sobreexpresados y 3 genes infraexpresados de manera estadísticamente significativa en los casos de LMA y reordenamiento *MYST3-CREBBP* (tabla 1 del trabajo). Posteriormente, se realizó un test de ANOVA para comparar las siete categorías de LMA incluídas en el análisis cuantitativo, definidas por las anomalías moleculares (A, *MYST3-CREBBP*; B, *PML-RARA*; C, *RUNX1-RUNX1T1*, D, *CBFB-MYH11*, E, t-*MLL*, F, *MLL*-PTD, G, otros). El test de ANOVA mostró cinco perfiles de expresión génica diferentes: en el primero (figura 20a y figura 2A del trabajo) se observa un grupo de 9 genes (*PRL, C20orf103, RET, GGA2, ICSBP1, ITGA7, □AP, IRAK1* y *PPARG*) con una elevada expresión en los casos de LMA-*MYST3-CREBBP* y una baja o nula expresión en el resto de pacientes. El segundo perfil (figura 20b y figura 2B del trabajo), muestra un alto nivel de expresión de 6 genes (*FLT3, HOXA9, MEIS1, AKR7A2, CH□3* y *APBA2*) en el grupo de LMA-*MYST3-CREBBP* y también en las leucemias de diferenciación monocítica. El tercer perfil observado (figura 20c y figura 2C del trabajo) incluía genes que presentaron una elevada expresión en los casos de LMA-*MYST3-CREBBP* y una expresión variable en el resto de muestras.

Figura 20. Estudio de la expresión relativa de genes mediante RT-PCR cuantitativa utilizando arrays de baja densidad en 40 pacientes con LMA. ▫iferentes perfiles de expresión génica observados en el análisis de ANOVA.

20a. Perfil 1. Genes sobreexpresados específicamente en las muestras de LMA-MYST3-CREBBP, con baja o nula expresión en el resto de casos.

20b. Perfil 2. Genes sobreexpresados en los subgrupos de LMA-MYST3-CREBBP y en los casos de diferenciación monocítica.

20c. Perfil 3. Genes sobreexpresados en la LMA-MYST3-CREBBP con una expresión variable en el resto de categorías.



Otros genes (*CCN□2, STAT5A*, *STAT5B* y *CREBBP)* estaban infraexpresados en la LMA-*MYST3-CREBBP* (perfil 4, figura 3A del trabajo). Finalmente, se observó una baja o nula del gen *WT1* tanto en la LMA-*MYST3-CREBBP* como en el subgrupo *RUNX1-RUNX1T1* (perfil 5, figura 3B del trabajo).

Por tanto, el análisis cuantitativo confirmó la existencia de un perfil génico específico de las LMA-*MYST3-CREBBP*, con la sobreexpresión de *PRL, C20orf103, RET, GGA2, ICSBP1, ITGA7, □AP, IRAK1* y *PPARG* y la infraexpresión de *CCN□2, STAT5A* y *STAT5B*. Por otro lado, se observó una similitud en la expresión de los genes *Flt3, HOXA9, MEIS1, AKR7A2, CH□3, Flt3, HOXA9, MEIS1, AKR7A2, CH□3* y *APBA2* en las LMA-*MYST3-CREBBP* y los casos de LMA con diferenciación monocítica, particularmente en los casos con reordenamiento del gen *MLL*.

Además, se observó un perfil de expresión específico de los genes *homeobox* en las LMA-*MYST3-CREBBP*¸ de forma que estas leucemias sobreexpresaban los genes *HOX*-5' (*HOXA9, HOXB9* y *HOXA10*), mientras que el resto de *HOX* se encontraban infraexpresados. Por el contrario, las LMA monocíticas y aquellas con reordenamiento del gen *MLL* presentaban una sobreexpresión global de los genes *HOX*¸ en contraste con los subgrupos de LMA de buen pronóstico, en los cuales la expresión de estos genes fue baja (ver figura 4 del trabajo).

Como resumen, con este trabajo se pudo establecer una firma génica específica para las leucemias con reordenamiento *MYST3-CREBBP*, consistente en la sobreexpresión de determinados genes *HOX* (*HOXA9, HOXA10*), de los oncogenes *RET* y *PRL* y la infraexpresión de genes como *CCN□2, STAT5* y *WT1*. Por otro lado, se observó una similitud en la expresión de algunos genes entre las leucemias *MYST3-CREBBP* y las LMA con reordenamiento de *MLL*, lo que sugiere un mecanismo de leucemogénesis parcialmente compartido por los dos tipos de leucemia.

Gene Expression Profiling of Acute Myeloid Leukemia with Translocation t(8;16)(p11;p13) and *MYST3-CREBBP* Rearrangement Reveals a Distinctive Signature with a Specific Pattern of *HOX* Gene Expression

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Abstract

Acute myeloid leukemia (AML) with translocation t(8;16)(p11;p13) is an infrequent leukemia subtype with characteristic clinicobiological features. This translocation leads to fusion of *MYST3* (*MOZ*) and *CREBBP* (*CBP*) genes, probably resulting in a disturbed transcriptional program of a myelomonocytic precursor. Nonetheless, its gene expression profile is unknown. We have analyzed the gene expression profile of 23 AML patients, including three with molecularly confirmed *MYST3-CREBBP* fusion gene, using oligonucleotide U133A arrays (Affymetrix). *MYST3-CREBBP* cases clustered together and clearly differentiated from samples with *PML-RARα*, *RUNX1-RUNX1T1*, and *CBFβ-MYH11* rearrangements. The relative expression of 46 genes, selected according to their differential expression in the high-density array study, was analyzed by low-density arrays in an additional series of 40 patients, which included 7 *MYST3-CREBBP* AML cases. Thus, genes such as prolactin (*PRL*) and proto-oncogene *RET* were confirmed to be specifically overexpressed in *MYST3-CREBBP* samples whereas genes such as *CCND2*, *STAT5A*, and *STAT5B* were differentially underexpressed in this AML category. Interestingly, *MYST3-CREBBP* AML exhibited a characteristic pattern of *HOX* expression, with up-regulation of *HOXA9*, *HOXA10*, and cofactor *MEIS1* and marked down-regulation of other homeobox genes. This profile, with overexpression of *FLT3*, *HOXA9*, *MEIS1*, *AKR7A2*, *CHD3*, and *APBA2*, partially resembles that of AML with *MLL* rearrangement. In summary, this study shows the distinctive gene expression profile of *MYST3-CREBBP* AML, with overexpression of *RET* and *PRL* and a specific pattern of *HOX* gene expression. (Cancer Res 2006; 66(14): 6947-54)

Introduction

Chromosomal translocations resulting in fusion proteins are a common finding in acute myeloid leukemia (AML). The most frequent fusion products, *PML-RARα*, *RUNX1-RUNX1T1* (*AML1-ETO*), and *CBFβ-MYH11*, found in ~25% of *de novo* AML cases, constitute abnormal transcriptional factors causing a disturbed program of myeloid differentiation. Moreover, each of these translocations defines a specific leukemia subtype associated with a favorable prognosis (1, 2). In this setting, translocation t(8;16)(p11;p13) is an infrequent recurrent chromosomal abnormality found in both *de novo* and therapy-related AML cases after treatment with topoisomerase II inhibitors (3–6). These patients present with specific clinical and biological features, such as a blast population with a myelomonocytic stage of differentiation, frequent extramedullary involvement, severe coagulation disorder, and a poor outcome (3, 5, 6). At the molecular level, translocation t(8;16) fuses MYST histone acetyltransferase (monocytic leukemia)-3 (*MYST3*; formerly named *MOZ*) and CREB binding protein (Rubinstein-Taybi syndrome; *CREBBP*, or *CBP*) genes, both encoding proteins with histone acetyltransferase activity (4, 7–9). *MYST3* has been shown to modulate gene transcription through activation of the transcription factor complex *RUNX1* (8, 10). Moreover, the protein complex MYST3-RUNX1 has been found to increase during normal monocytic differentiation. In its turn, CREBBP protein also regulates transcription by means of histone acetyltransferase activity and by binding to several proteins with key cell cycle functions, such as p53 and nuclear factor κB (8, 9). Therefore, an inhibition of *RUNX1*-mediated transcription by MYST3-CREBBP fusion protein has been hypothesized to be the main mechanism of leukemogenesis in this AML variety (10). However, the precise pathways disrupted by this chimerical protein are mostly unknown.

Analysis of gene expression profile might contribute to refine the classification of AML based on biological grounds and to assign the prognostic risk of a given subtype more accurately (11–14). Furthermore, genomic analysis of AML might provide a deeper insight into the underlying disease mechanisms.

In this study, we have examined the gene expression profile of AML with the *MYST3-CREBBP* fusion gene to determine the specific signature of this leukemia compared with other well-defined AML subtypes and to define possible molecular pathways involved in the pathogenesis of this leukemia.

Materials and Methods

Leukemia samples. Twenty-three AML patients were selected for a global gene expression profile analysis using the Affymetrix HU133A array (Affymetrix, Inc., Santa Clara, CA; subset A of patients, Supplementary Table A). These cases included three *MYST3-CREBBP* AML cases, together with other 20 samples of different leukemia subtypes classified according to WHO criteria (15) as acute promyelocytic leukemia with t(15;17)(q22;q12) (*PML-RARα*; $n = 3$); AML with t(8;21)(q22;q22) (*RUNX1-RUNX1T1*; $n = 3$); AML with inv(16)/t(16;16) (*CBFβ-MYH11*; $n = 3$); AML with t(9;11)(p22;q23) (*MLLT3-MLL*; $n = 1$); acute monocytic leukemias ($n = 8$); and two cases of AML with multilineage dysplasia. In 10 of these 23 cases, an internal tandem

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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duplication of the *FLT3* gene was detected whereas mutations of nucleophosmin (*NPM*) gene were found in 6 cases (Supplementary Table A). Two cases with *MYST3-CREBBP* rearrangement and one monocytic-differentiated AML (cases 1, 3, and 18) followed exposure to topoisomerase II inhibitors. Cases 1 and 18 also presented amplification of *MLL* gene.

To confirm the findings of the previous global gene expression profile study, a second subset of 40 AML patients (subset B, Supplementary Table B) was studied using TaqMan low-density arrays (Applied Biosytems, Foster City, CA; see below). This subset of patients included the three *MYST3-CREBBP* AML cases previously studied by high-density array and four additional *MYST3-CREBBP* samples with no appropriate material for the genome-wide assay. In addition, an independent set of 33 AML samples was included in this study. These samples corresponded to AML with well-characterized rearrangements (*PML-RARα, n* = 3; *RUNX1-RUNX1T1, n* = 3; *CBFβ-MYH11, n* = 3; *MLL*-rearranged AML, *n* = 9) and normal karyotype AML (*n* = 15). Mutations of *FLT3-ITD, NPM*, and *MLL* abnormalities are detailed in Supplementary Table B. None of the *MYST3-CREBBP* samples analyzed harbored either *FLT3-ITD* or *NPM* mutations. Four patients (cases 2, 3, 7, and 18) presented with a therapy-related AML. The clinical and laboratory data of the seven *MYST3-CREBBP* AML cases were previously reported (16). All peripheral blood and bone marrow samples were obtained with informed consent according to the guidelines of the Ethical Committee of the participating Institutions.

RNA extraction and cDNA synthesis. Total RNA was isolated from peripheral blood and bone marrow by standard methods (17). In one case, we obtained RNA from a paraffin-embedded tissue using a phenol-extraction method. In all cases, integrity of RNA was examined with Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). One microgram of RNA was reverse transcribed to cDNA using random primers with the High Capacity cDNA Archive Kit (Applied Biosystems).

Molecular analyses. The detection of transcript type I of *MYST3-CREBBP* rearrangement was analyzed by reverse transcription-PCR (RT-PCR) with primers MOZ3558F and CPB335R, as previously described (16). The presence of *PML-RARα, RUNX1-RUNX1T1*, and *CBFβ-MYH11* rearrangement was analyzed by RT-PCR following published conditions (18). The analysis of *FLT3-ITD* was done using primers and conditions previously published (19). The presence of mutations in exon 12 of *NPM* gene was studied by amplification of genomic DNA or cDNA with a fluorescently labeled forward primer and subsequent analysis of the PCR product in an Automatic sequencer (Abi Prism 310) using the Genescan software as described (20). *MLL* rearrangement was studied by Southern blot analysis using probe B859 after digestion with *Hind*III and *Bam*HI enzymes (21). Cases without available DNA, with a noninformative karyotype, or showing abnormalities at 11q23 region by conventional cytogenetics were studied by fluorescence *in situ* hybridization with the LSI *MLL* Dual Color Probe (Vysis) as described (22). Partial tandem duplication was specifically analyzed by RT-PCR as previously described (23).

High-density array study: RNA purification, labeling, and hybridization. In this part of the study, 23 AML samples with confirmed high quality RNA by Agilent 2100 Bioanalyzer analysis and sufficient amount of RNA (5 μg) were included. Amplified biotinylated complementary RNA was produced with an *in vitro* transcription labeling reaction and was subsequently hybridized to Affymetrix HU133A oligonucleotide arrays following the Affymetrix protocol for high-density arrays (details are provided in supplementary material). Scans were carried out on Agilent G2500A GeneArray scanner (Agilent Technologies, Waldbronn, Germany) and the fluorescence intensities of scanned arrays were analyzed with the Affymetrix GeneChip software.

Statistical analysis. Affymetrix Microarray Suite Software version 5.0 (MAS5.0) was used for the quantification of the expression level of target genes. HU133A microarray raw expression intensities were scaled to a target intensity of 200 units. To exclude genes with minimal variation across samples, only genes with a mean (SD) of normalized values between 0.7 and 10 were filtered. Thereafter, we selected those genes with an expression level of ≥20 in ≥25% of samples. The 2,683 resulting genes were studied by means of unsupervised two-dimensional cluster analysis with dChip v1.3 software using the default clustering algorithm defined as $1 - r$, where r is the Pearson correlation coefficient between standardized expression values (make 0 and SD 1) and the centroid linkage method. To identify those genes with significant differences in their expression level among different AML categories, we used a random-variance F test as described in BRB ArrayTools software (BRB ArrayTools developed by Dr. Richard Simon and Amy Peng Lam)[5] with all probe sets, but assigning an arbitrary value of 10 to genes with an expression level below 10 units. A significance level of 0.001 was chosen to reduce the number of false positive results. For each one of the differentially expressed genes, a ratio between the mean expression value in *MYST3-CREBBP* samples and the mean expression in every AML category was calculated. Additionally, a t test comparing the gene expression in *MYST3-CREBBP* and that in the remaining samples as a whole, with a significance level of 0.001, was used as another method of detection of differentially expressed genes in *MYST3-CREBBP* samples.

Quantitative real-time RT-PCR. A selection of 46 genes was subsequently studied by real-time PCR using TaqMan Low Density Arrays (Applied Biosystems) in an additional series of 40 AML patients (subset B, Supplementary Table B). These genes were selected on the basis of their differential expression in *MYST3-CREBBP* according to high-density array analysis or their oncogenic potential in leukemia. The list of 46 genes is provided in Supplementary Table C. Briefly, cDNA was obtained from these cases, loaded onto the low-density arrays, and amplified using standard conditions in an Abi Prism 7900HT Sequence Detection System (Applied Biosystems). All samples were tested in duplicate and the average value between replicates was taken as the specific level of expression of a given gene. To quantify the relative expression of each gene, the Ct values were normalized for endogenous reference ($\Delta Ct = Ct_{target} - Ct_{\beta 2\text{-microglobulin}}$) and compared with a calibrator using the $\Delta\Delta Ct$ method. As calibrator, the average Ct value of each gene in all samples grouped together was taken. Comparison of the relative expression of the 46 genes in *MYST3-CREBBP* AML with that in the remaining AML samples was done using a t test comparing two groups, with a significance level of 0.05. In addition, an ANOVA test was also used to compare the relative expression of these genes after defining different AML categories (significance level, 0.05).

Results

High-density Array Analysis

Unsupervised analysis. After applying a variation filter, the resulting 2,683 genes were visualized by hierarchical clustering method (Fig. 1*A*). Two main branches were seen in the dendrogram, corresponding mainly to cases with myeloid and monocytic differentiation, respectively. Samples clearly grouped in five clusters, which were constituted by cases of AML with well-defined gene rearrangements [i.e., *MYST3-CREBBP* (cluster 1), *RUNX1-RUNX1T1* (cluster 2), *CBFβ-MYH11* (cluster 3), and *PML-RARα* (cluster 4), and, on the other hand, AML with multilineage dysplasia (cluster 5)]. In contrast, the remaining samples (no. 13-21), defined by their monocytic differentiation and the absence of the above-mentioned fusion proteins, were distributed among the different clusters of the array forming a heterogeneous group. One of the samples harboring a *CBFβ-MYH11* rearrangement, with a minimally differentiated phenotype (M0 subtype), segregated from two other cases with the same molecular alteration but presenting with a myelomonocytic phenotype.

Supervised analysis. We applied supervised methods based on the six different AML categories (clusters 1-5 and a sixth group containing monocytic-lineage leukemias) drawn from the unsupervised analysis. First, the analysis with a random-variance F test yielded 1,205 genes with a significant different expression level among AML subgroups. A hierarchical cluster done with this set of genes is presented in Fig. 1*B*. Afterwards, we selected 63 genes

[5] http://linus.nci.nih.gov/BRB-ArrayTools.html.

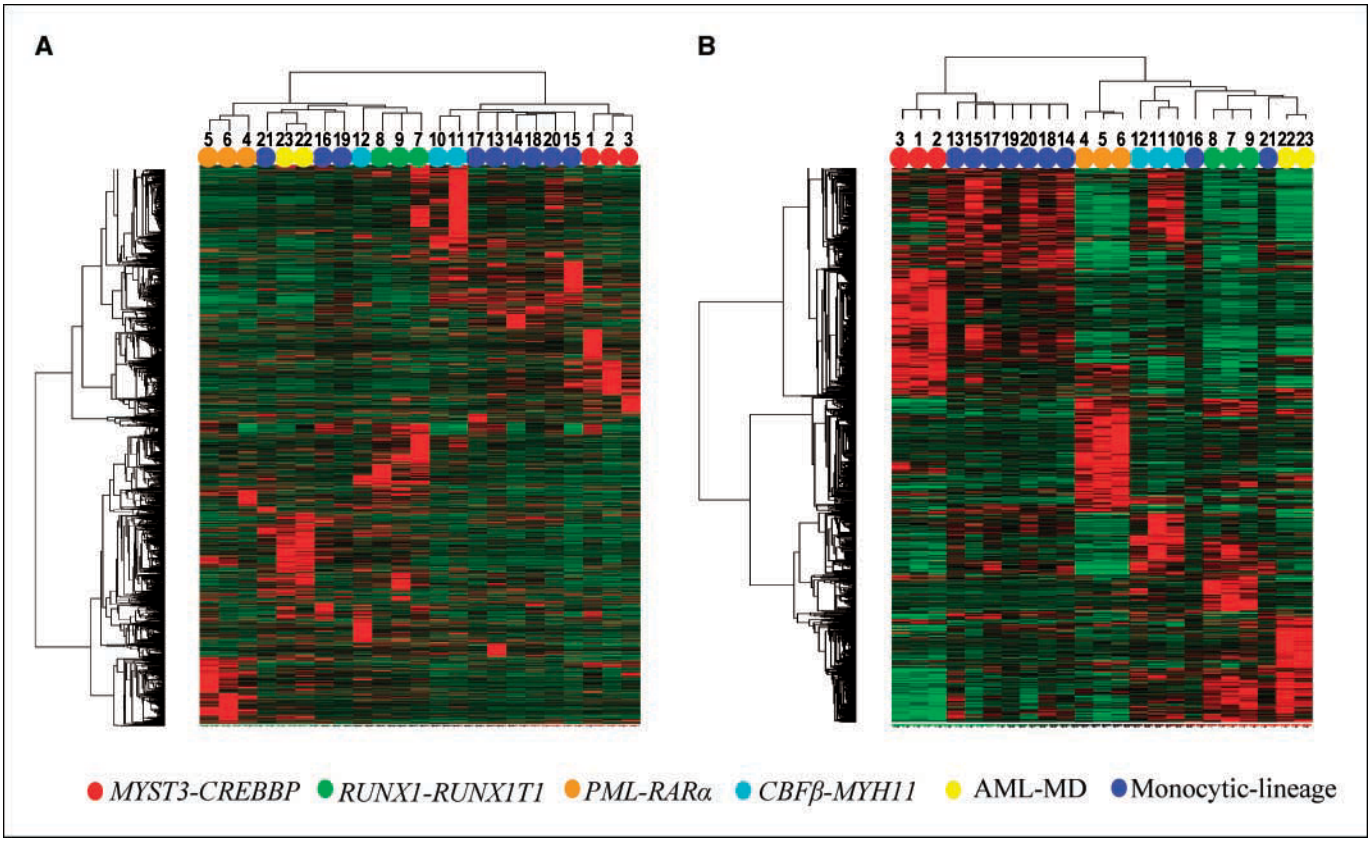

Figure 1. High-density array study done in 23 AML cases (subset A of patients). *A,* visualization of the results of the unsupervised analysis. The 2,683 genes obtained after applying a variation filter were visualized by hierarchical clustering method. Two main branches were distinguished according basically to the myeloid or monocytic differentiation lineage of blast cells. Five clusters were recognized, constituted by cases with well-defined rearrangement [i.e., *MYST3-CREBBP* (cluster 1), *RUNX1-RUNX1T1* (cluster 2), *CBFβ-MYH11* (cluster 3), and *PML-RARα* (cluster 4), and, on the other hand, AML with multilineage dysplasia (cluster 5)]. The remaining samples (nos. 13-21), defined by their monocytic differentiation and the absence of the above-mentioned fusion proteins, were distributed among the different clusters of the array forming a heterogeneous group. *B,* visualization of genes with a differentiated expression in the different AML subgroups. Hierarchical clustering visualization of the 1,205 genes with a significant different expression level ($P \leq 0.01$) among the six different AML categories (clusters 1-5 and a sixth group containing monocytic-lineage leukemias) using a random-variance *F*-test method.

overexpressed and 60 genes underexpressed in *MYST3-CREBBP* samples with a ratio of mean expression equal or higher than twice the observed in each of the other groups. The 63 genes specifically overexpressed in *MYST3-CREBBP* samples (Supplementary Table D; Supplementary Fig. *A*1) included the oncogene *RET*, genes involved in chromatin remodeling and transcription (*HOXA10* and *PPARG*), and genes with a known function in DNA damage repair (*DDB2*) and apoptosis (*DAP*). Other genes such as *IRAK1*, expressed in response to cell injury, *NICAL*, implied in neuronal development, and prolactin (*PRL*), involved in signal transduction, were also up-regulated. In addition, 60 genes specifically down-regulated in *MYST3-CREBBP* leukemias (Supplementary Table D; Supplementary Fig. *A*2) included genes involved in cell cycle regulation, such as cyclin D2 (*CCND2*) and two members of the RAS oncogene family (*RAB6A* and *RAB8A*).

As an additional method to study possible shared patterns of gene expression between *MYST3-CREBBP* and other AML, we selected those genes with high or low expression in *MYST3-CREBBP* leukemias and a similar pattern of expression in one of the other AML categories (Supplementary Tables E and F; Supplementary Fig. *B*). This analysis revealed that *MYST3-CREBBP* leukemias had 33 genes overexpressed in common with AML of monocytic lineage (i.e., *AKR7A2, CHD3*, and *AK2*) and 19 with *PML-RARα*, but only a minority of genes in common with other AML subtypes.

Finally, when *MYST3-CREBBP* cases were compared with the other samples as a group using a *t* test, 237 genes (53 overexpressed and 184 underexpressed) showed a differential expression (Supplementary Tables G and H). Using this analysis, a high expression of the aforementioned gene *RET* could be observed. Additionally, an overexpression of genes with a role in transcription (*SATB1*), genes involved in apoptosis (*OPTN*), and *CEBPα*, a crucial gene in the myeloid differentiation process, was observed.

Low-density Array Analysis

Supervised analysis. Forty-six genes were selected according to their differential expression in *MYST3-CREBBP* samples in the high-density array and/or their relevant oncogenic role in leukemia. The relative expression of these genes was analyzed in 40 AML patients (subset B, Supplementary Table B). To define the group of genes characteristic of *MYST3-CREBBP* subtype, a *t* test with two groups was used to compare the gene expression in *MYST3-CREBBP* samples and the remaining AML patients. Twenty-two genes were significantly overexpressed in *MYST3-CREBBP* samples whereas three genes were underexpressed in this group of leukemias (Table 1). Subsequently, an ANOVA test was used to compare the gene relative expression among the seven AML categories defined by their underlying molecular abnormality (A, *MYST3-CREBBP*; B, *PML-RARα*; C, *RUNX1-RUNX1T1*; D, *CBFβ-MYH11*;

Table 1. Genes with a significant differential expression level (overexpressed and underexpressed) in *MYST3-CREBBP* samples according to the low-density array study

t test		ANOVA	
Symbol	*P*	Symbol	*P*
Genes overexpressed			
C20ORF103	0.000000	*C20ORF103*	0.000000
GGA2	0.000000	*ICSBP1*	0.000010
ICSBP1	0.000000	*FLT3*	0.000087
FLT3	0.000000	*GGA2*	0.000094
LMO2	0.000005	*AKR7A2*	0.000245
ITGA7	0.000013	*SURF1*	0.002103
AKR7A2	0.000014	*LMO2*	0.002979
PPARG	0.000016	*HIST1H2A*	0.004580
SURF1	0.000024	*ITGA7*	0.005785
ADAMTS2	0.000036	*PPARG*	0.006282
DAP	0.000092	*ADAMTS2*	0.014015
IRAK1	0.000093	*CEBPA*	0.014782
PRL	0.000096	*IRAK1*	0.019370
HIST1H2A	0.000134	*LGALS3*	0.021620
RET	0.000260	*DDB2*	0.023266
CHD3	0.000264	*DAP*	0.025509
LGALS3	0.000749	*PRL*	0.027868
DDB2	0.000829	*SATB1*	0.039598
SATB1	0.001823		
HOXA9	0.003608		
S100A11	0.015956		
CEBPA	0.021236		
Genes underexpressed			
STAT5B	0.036902	*WT1*	0.000061
CREBBP	0.041165	*CCND2*	0.001073
STAT5A	0.049642	*STAT5B*	0.003404
		STAT5A	0.027617

E, *MLL*-translocated samples; F, *MLL* partial tandem duplication; and G, miscellaneous). The ANOVA test yielded five different gene expression profiles. First (profile 1, Fig. 2*A*), a group of nine genes (*PRL, C20orf103, RET, GGA2, ICSBP1, ITGA7, DAP, IRAK1* and *PPARG*) were found to be highly expressed in *MYST3-CREBBP* AML with absent or low expression in the remaining samples. Genes such as *FLT3, HOXA9, MEIS1, AKR7A2, CHD3*, and *APBA2* composed a second pattern of expression (profile 2, Fig. 2*B*), with high expression level in both *MYST3-CREBBP* and AML with monocytic phenotype. In the third profile observed (profile 3, Fig. 2*C*), some genes had a high expression level in *MYST3-CREBBP* AML and were variably expressed in other AML categories. This group included *SATB1, LMO2, CEBPα, SURF1, HIST1H2A, DDB2*, and *LGALS3* genes.

Another group of genes were characteristically down-regulated in the *MYST3-CREBBP* leukemia subtype compared with other AML categories, including *CCND2, STAT5A*, and *STAT5B* (profile 4, Fig. 3*A*). A decrease in expression levels of *CREBBP* was seen in *MYST3-CREBBP* subtype, but the difference did not reach statistical significance in the ANOVA test comparing the AML subgroups. Finally, a low or absent expression of Wilms' tumor 1 (*WT1*) gene was distinctly observed in both *MYST3-CREBBP* and *RUNX1-RUNX1T1* cases (profile 5, Fig. 3*B*).

Mutational Status of *RET* Gene

Due to the distinctly overexpression of *RET* gene observed in *MYST3-CREBBP* samples, mutations of this gene were screened by direct sequencing of exons 8 to 16, where somatic and germ-line mutations associated with human diseases have previously been described (24). None of the five *MYST3-CREBBP* samples that could be analyzed harbored mutations of *RET* gene, and only neutral polymorphisms at codons 769 and 836 were found (data not shown).

Expression of Homeobox Genes

Given the high expression of several homeobox (*HOX*) genes observed in *MYST3-CREBBP*, we did an unsupervised analysis on the whole series of patients focused on the relative level of expression of the *HOX* genes present in the array. Several patterns of *HOX* genes expression were seen in different AML categories (Fig. 4*A*). Thus, overexpression of 5′-*HOX* genes (*HOXA9, HOXB9*, and *HOXA10*) was characteristically found in all *MYST3-CREBBP* samples and most cases of monocytic AML. Other *HOX* genes (*HOXA2-A7* and *HOXB2-B7*) showed a high expression in the group of monocytic leukemias whereas their expression was low in *MYST3-CREBBP* AML. In contrast, *HOX* genes were underexpressed in good-risk cytogenetic AML.

Ten genes of the *HOX* family were subsequently studied by low-density arrays. With this method, the distinctive pattern of *HOX* expression according to AML subtypes was confirmed (Fig. 4*B*). Thus, good-prognosis AML showed a lower expression of all *HOX* analyzed. On the contrary, a high expression of all the *HOX* genes studied was detected in *MLL*-rearranged, partial tandem duplication, and monocytic-phenotype AML. In addition, *MYST3-CREBBP* AML showed a high expression of *HOXA9* and *MEIS1* but a lower expression of the remaining *HOX* genes.

Discussion

AML with *MYST3-CREBBP* rearrangement is an infrequent leukemia subtype resulting from the fusion of two genes with chromatin-modifying properties. In this regard, inhibition of *RUNX1*-mediated transcription, possibly through a disturbed histone acetyltransferase activity, has been hypothesized to be the main mechanism of leukemogenesis (10). Nevertheless, the signaling pathways disrupted by the chimerical protein MYST3-CREBBP are mostly unknown and, to the best of our knowledge, no previous studies have focused on the genomic profile of this AML variety. With this purpose, we used high-density microarrays to study a series of 23 AML samples that included three *MYST3-CREBBP* cases. The unsupervised analysis of the results identified a distinctive gene expression signature associated with the *MYST3-CREBBP* rearrangement. Thereafter, a group of genes was selected and analyzed using a quantitative approach with low-density arrays. This technology allowed us to study four additional *MYST3-CREBBP* samples with no appropriate material for the genome-wide assay. Interestingly, this approach confirmed the existence of a characteristic gene expression profile in *MYST3-CREBBP* AML, clearly distinguishable from that of other well-defined AML subtypes.

The combination of several methods of comparative analysis allowed the identification of groups of genes with a differential expression in distinct AML categories. First, a subset of genes seemed to be highly characteristic of *MYST3-CREBBP* AML, being up-regulated in these cases and showing a low or absent expression

in the remaining AML categories. Thus, genes such as *PRL, C20orf103, RET, GGA2, ICSBP1, ITGA7, DAP, IRAK1*, and *PPARG* were overexpressed almost exclusively in *MYST3-CREBBP* cases. Among those genes, *PRL* and *RET* have been occasionally reported to be involved in leukemogenesis (24–33). In this regard, an increased expression of prolactin protein in blast populations has been observed in anecdotal cases of monocytic-lineage leukemia as well as in the eosinophilic cell line Eol-1 (25–29). In a recent study, prolactin expression in Eol-1 cells was shown to involve different signaling pathways induced by cyclic AMP whereas inhibition of Jak-STAT5 pathway resulted in up-regulation of prolactin (28). Interestingly, *STAT5A* and *STAT5B* genes were found to be significantly underexpressed in our *MYST3-CREBBP* AML samples, suggesting a negative regulatory effect between prolactin and STAT5 proteins. On its turn, *RET* gene is a proto-oncogene that encodes a tyrosine kinase receptor expressed during normal myelomonocytic differentiation and, accordingly, has been found to be predominantly expressed in AML of monocytic phenotype (30–32). Moreover, RET mRNA levels are typically low among immature $CD34^+$ hemopoietic progenitors whereas overexpression

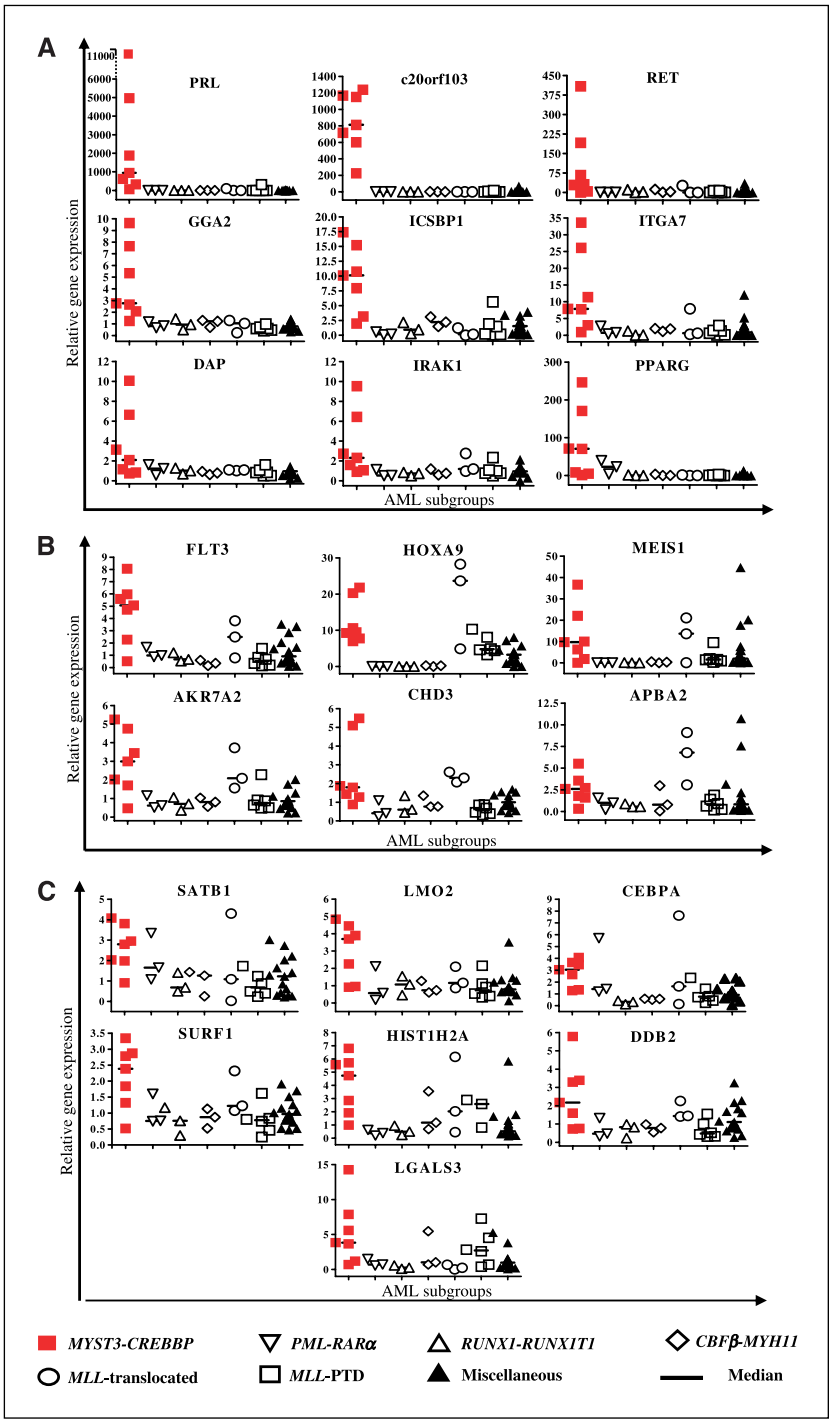

Figure 2. Low-density array study: genes significantly overexpressed in *MYST3-CREBBP* samples (ANOVA test). *A,* profile 1. Genes specifically overexpressed in *MYST3-CREBBP* samples, with absent or low expression in the remaining AML categories. *B,* profile 2. Genes overexpressed in *MYST3-CREBBP* and monocytic AML, either *MLL*-rearranged or non-rearranged variants. *C,* profile 3. Genes overexpressed in *MYST3-CREBBP* samples with variable expression in other AML categories.

of this protein has been associated with coexpression of adhesion molecules such as CD56 (30). These observations resemble the characteristic phenotype of *MYST3-CREBBP* AML, defined by common CD34 negativity, high expression of monocytic antigens, and frequent coexpression of CD56 and NG2 (16). Of note, *RET* has recently been reported as one of the most characteristic genes in one of the 16 AML clusters defined according to its genomic profile in a recent study by Valk et al. (14). Although most of the cases forming that cluster were monocytic-differentiated leukemias (14), modulation of *RET* does not seem to be merely attributable to a monocytic differentiation process because, in the present study, the expression level of *RET* was significantly higher in *MYST3-CREBBP* cases than in other monocytic-lineage leukemia samples. Somatic and germ-line *RET* mutations leading to gene activation are responsible for several human diseases, including multiple endocrine neoplasia types 2A and 2B and papillary thyroid carcinomas (24). Nevertheless, mutations of *RET* as a mechanism of overexpression were discarded in the present *MYST3-CREBBP* series, in accordance with a previous study analyzing diverse AML subtypes (33).

One of the most striking findings of this study was the similarities observed between *MYST3-CREBBP* and *MLL*-rearranged leukemias. *MYST3-CREBBP* cases presented high levels of homeobox genes (*HOXA9* and *HOXA10*), their cofactor *MEIS1*, and the receptor with tyrosine-kinase activity *FLT3*, all of them typically up-regulated in *MLL* leukemias (34–36). *HOX* genes are transcription factors required for a proper hematopoietic development and constitute downstream targets of *MLL* protein (37). In this regard, *MLL*-rearranged leukemias are typically characterized by an impaired pattern of *HOX* expression, and *HOXA9, HOXA10*, and the cofactor *MEIS1* are up-regulated in virtually all lymphoid, myeloid, and biphenotypic lineage *MLL*-rearranged leukemia subtypes (38). In addition to the unexpected *HOX* overexpression in *MYST3-CREBBP* cases, the results of the present study confirmed previous findings on the pattern of *HOX* expression in different AML subtypes (39–41). Thus, whereas an overall low expression of *HOX* genes was observed in cases of AML with favorable cytogenetics, high levels of the members of *HOX* family *HOXA9* and *HOXA10* were seen in *MYST3-CREBBP* and *MLL*-rearranged leukemias. In contrast, other *HOX* genes analyzed, such as *HOXA3, HOXA7*, and *HOXB5*, were expressed at different levels in *MLL*-translocated cases, *MLL* with partial tandem duplication, and other monocytic leukemias, but were not expressed in *MYST3-CREBBP* AML cases.

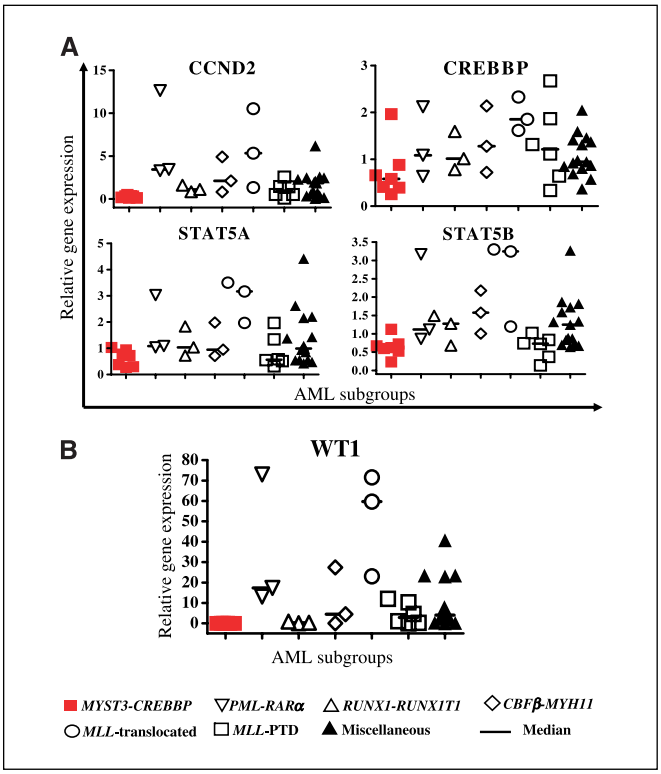

Figure 3. Low-density array study: genes significantly underexpressed in *MYST3-CREBBP* samples (ANOVA test). *A,* profile 4. Genes specifically underexpressed in *MYST3-CREBBP* samples. *B,* profile 5. *WT1* gene was found to be characteristically underexpressed in *MYST3-CREBBP* samples as well as in *RUNX1-RUNX1T1* cases.

Similarities between *MYST3-CREBBP* and *MLL* leukemias comprised other genes. Thus, overexpression of A*KR7A2, PBX3, NICAL*, and *IRAK1B* genes, observed in *MYST3-CREBBP* subtype, was also found in AML with *MLL* rearrangement, as reported in a recent study by Kohlmann et al. (36). Moreover, a coincidental expression of several genes (*RET, C20orf103, GGA2, GAGED2, AKR7A2*, and *AK2*) was observed between our *MYST3-CREBBP* samples and the above-mentioned cluster no. 16 of the study of Valk et al. (14). Of note, 5 of the 11 patients included in this cluster had 11q23 abnormalities.

As a common mechanism of leukemogenesis, chimeric protein *PML-RARα* in acute promyelocytic leukemia and *RUNX1-RUNX1T1* and *CBFβ-MYH11*, associated to core binding factor leukemias, respectively, induce a constitutive transcriptional repression, leading to a blockage of the normal myeloid differentiation program (42). This contrasts with the presumed function of fusion gene products derived from the rearrangement of *MLL* with different partners, thought to produce a constitutive transcriptional activation through a gain-of-function mechanism resulting in an inappropriate expression of target genes such as *HOX* (42). In this context, the gene expression signature of *MYST3-CREBBP* rearrangement obtained in this study seemed to be similar to that of *MLL*-rearranged leukemia and markedly different from those of CBF-AML and acute promyelocytic leukemia. Therefore, the leukemogenic effect of *MYST3-CREBBP* fusion gene could rely on a deregulated modulation of downstream targets, resembling that of *MLL* chimeras, probably due to impaired histone acetyltransferase activity of the proteins involved in this translocation. Moreover, the adverse prognosis classically associated with this entity differs from that of CBF-AML and acute promyelocytic leukemia and is similar to that of *MLL*-rearranged AML.

With regard to the underexpressed genes, the *CREBBP* expression was decreased in *MYST3-CREBBP* samples, although not reaching a significant difference, thus suggesting a negative regulation of the chimerical protein *MYST3-CREBBP* over native *CREBBP* transcript. Additionally, down-regulation of *WT1* was observed in *MYST3-CREBBP*, as well as in *RUNX1-RUNX1T1*, as compared with the other AML categories. *WT1* gene has been shown to be overexpressed in >90% of AML cases and it has recently been proposed as a molecular marker for minimal residual disease studies in AML (43). However, the low levels of *WT1* in *MYST3-CREBBP* leukemias observed in our study would hamper this strategy in the follow-up of the minimal residual disease in this group of leukemia.

In summary, the double strategy followed, based on the selection of a group of genes according to their differential expression in the high-density array assay and further analysis by real-time PCR in an additional set of patients, allowed the

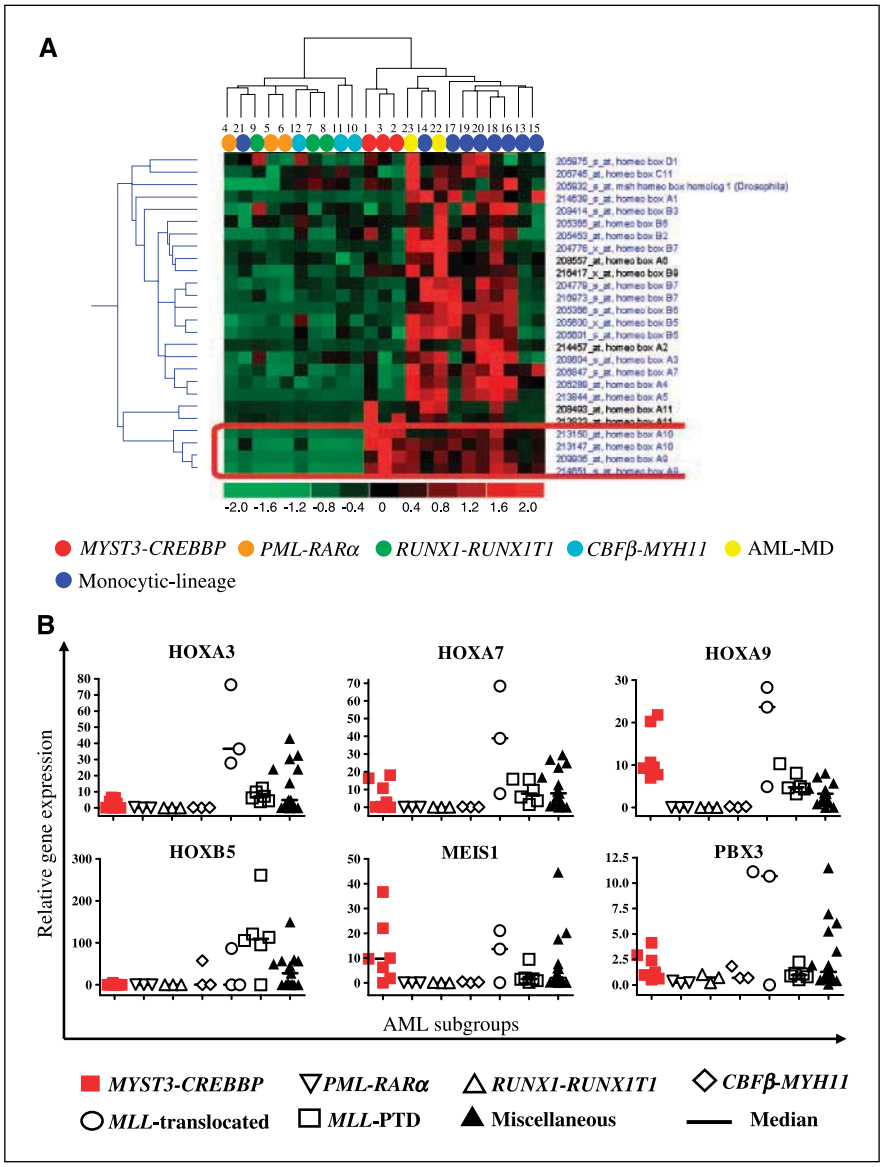

Figure 4. Gene expression signature of homeobox genes. *A,* high-density array study. Visualization of the homeobox genes in the unsupervised analysis using hierarchical clustering method. Several 5′-homeobox genes (*HOXA9, HOXB9*, and *HOXA10*) were overexpressed in *MYST3-CREBBP* samples and most cases of monocytic-lineage AML. In contrast, other *HOX* genes (*HOXA2-A7* and *HOXB2-B7*) showed a high expression level only in monocytic leukemias but not in *MYST3-CREBBP* samples. Overall, *HOX* expression was low in good-risk cytogenetic AML categories. *B,* low-density array study. Comparative expression of homeobox genes among the different AML categories using an ANOVA test confirmed the results of the high-density array study with high expression of *HOXA9* and *MEIS1* in *MYST3-CREBBP* cases and overall low expression in good-prognosis AML. In addition, *MLL*-rearranged cases showed the highest expression of all the *HOX* genes studied.

assessment of gene profile in a group of seven *MYST3-CREBBP* patients. Of note, a distinctive gene expression signature of *MYST3-CREBBP* leukemias was observed, characterized by the over-expression of homeobox genes *HOXA9, HOXA10*, and their cofactor *MEIS1*; the up-regulation of the oncogenes *RET* and *PRL*; and the decreased expression of genes such as *CCND2, STAT5*, and *WT1*. This profile harbors some similarities with that of *MLL*-rearranged leukemias, thus suggesting a partially common leukemogenic pathway.

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SUPPLEMENTARY MATERIAL: INDEX PAGE

SUPPLEMENTARY MATERIAL

Table A. Main characteristics of the subset of 23 AML patients studied by high-density arrays (subset A)

	Age/Gender	WHO / FAB subtype	Karyotype	WBC (x10^9/L)	BM blasts (%)	*MYST3-CREBBP*	*FLT3*-ITD	*NPM* status	*MLL* status
1	51 / F	M4	NA	21	78	+	-	G	A
2	28 / M	M4	t(8;16)(p11;p13)	8	92	+	-	G	G
3	53 / F	M4	t(8;16)(p11;p13)	27	96	+	NA	G	NA
4	24 / M	*PML-RARα* / M3	t(15;17)	5	90	-	-	G	G
5	52 / M	*PML-RARα* / M3	t(15;17)	1.5	75	-	-	G	G
6	31 / F	*PML-RARα* / M3	t(15;17)	3.2	89	-	-	G	G
7	23 / M	*RUNX1-RUNX1T1* / M2	t(8;21)	38	34	-	+	ND	G
8	52 / M	*RUNX1-RUNX1T1* / M2	t(8;21)	8	68	-	-	ND	G
9	40 / M	*RUNX1-RUNX1T1* / M2	t(8;21)	5.1	54	-	-	ND	G
10	63 / M	*CBFβ-MYH11* / M4	inv(16)	297	80	-	-	ND	G
11	63 / M	*CBFβ-MYH11* / M4	inv(16)	7	74	-	-	ND	G
12	59 / M	*CBFβ-MYH11* / M0	t(16;16)	108	94	-	-	ND	G
13	41 / F	*MLLT3-MLL* / M5	t(9;11)	51	90	-	+	G	R
14	38 / F	M5	46, XX	36	79	-	+	G	G
15	76 / M	M4	46 XY, der(10)	21	90	-	-	G	NA
16	59 / M	M4	NA	29	59	-	-	M	G
17	26 / M	M5	46, XY	295	92	-	+	G	G
18	62 / F	M5	NA	67	88	-	+	M	A
19	47 / F	M5	del(11q23)	17	78	-	+	M	G
20	50 / F	M5	46, XX	61	59	-	+	M	G
21	28 / F	M5	46, XX	132	90	-	+	G	G
22	30 / F	AML-MD / M5	46, XX	6	79	-	+	M	G
23	64 / M	AML-MD / M1	46, XY	17	83	-	+	M	G

WBC: white blood cell. BM: bone marrow. FLT3-ITD: *FLT3* Internal tandem duplication. NA: not available. A: amplification of *MLL* gene observed by FISH analysis. G: germinal. ND: not done. R: rearrangement of *MLL* gene confirmed either by Southern Blot or FISH analyses. M: mutated.

Table B. Main characteristics of the subset of 40 AML patients studied by real-time PCR using low-density arrays (subset B)

	Age/Gender	WHO / FAB subtype	Karyotype	WBC ($x10^9$/L)	BM blasts (%)	*MYST3-CREBBP*	*FLT3*-ITD	*NPM* status	*MLL* status
1	51 / F	M4	NA	21	78	+	-	G	A
2	28 / M	M4	t(8;16)(p11;p13)	8	92	+	-	G	G
3	53 / F	M4	t(8;16)(p11;p13)	27	96	+	-	G	NA
4	79 / M	M4	NA	16	63	+	-	NA	NA
5	19 / M	M4	t(8;16)(p11;p13)	14	70	+	NA	G	G
6	51/ F	M4	t(8;16)(p11;p13)	40	89	+	-	G	G
7	30 / F	M4	t(8;16)(p11;p13)	6	56	+	-	G	G
8	78 / M	*PML-RARα* / M3	t(15;17)	0.8	61	-	-	G	G
9	75 / M	*PML-RARα* / M3	t(15;17)	2.9	89	-	-	G	G
10	52 / F	*PML-RARα* / M3	t(15;17)	4.3	84	-	-	G	G
11	48 / M	*RUNX1-RUNX1T1* / M2	t(8;21)	54	86	-	-	ND	G
12	25 / M	*RUNX1-RUNX1T1* / M2	t(8;21)	54	24	-	-	ND	G
13	40 / M	*RUNX1-RUNX1T1* / M2	t(8;21)	6.2	63	-	-	ND	G
14	44 / M	*CBFβ-MYH11* / M4	inv(16)	1.2	45	-	-	ND	G
15	64 / M	*CBFβ-MYH11* / M4	inv(16)	16	48	-	-	ND	G
16	34 / M	*CBFβ-MYH11* / M4	Inv(16)	15	41	-	-	ND	G
17	44 / F	M5	NA	160	100	-	+	M	R
18	31 / F	M5	92, XXXX	0.5	97	-	-	G	R
19	45 / F	11q23 abn AML / M5	complex	130	95	-	-	G	R
20	54 / M	M5	46, XY	84	85	-	+	G	R- PTD
21	64 / F	M5	46, XX	97.1	66	-	+	G	R- PTD
22	61/ F	M4	46, XX	45.5	35	-	+	G	R- PTD
23	18 / F	M5	46, XX, der(1)	9	88	-	-	G	R- PTD
24	42 / F	M1	46, XX, 18p+	3.9	90	-	-	G	R- PTD
25	46 / M	M2	46, XY	3.5	22	-	-	G	R- PTD
26	52 / M	AML-MD / M2	46, XY	1.5	86	-	+	G	G
27	53 / M	AML-MD / M5	Complex	171	79	-	+	G	G
33	32 / M	AML-MD / M1	47, XY,+8	19.5	62	-	+	G	G
34	30 / F	AML-MD / M1	46, XX	6.3	35	-	+	M	G
28	54 / F	M5	46, XX	63	88	-	+	M	G
29	52 / M	M5	47, XY,+8	141	79	-	+	M	G
30	55 / F	M5	46, XX	140	32	-	-	M	G
31	46 / M	M5	46, XY	128	65	-	-	M	G
32	48 / M	M4	46, XY	179	88	-	-	G	G
35	28 / M	M2	Complex	5.1	33	-	-	G	NA
36	76 / M	M4	46, XY	21	90	-	-	G	NA
37	41 / F	M1	46, XX	1,2	53	-	-	G	G
38	60 / M	M1	46, XY	1.8	67	-	-	G	G
39	61 / F	M2	47, XX, +8, i(17q)	6	86	-	-	G	G
40	58 / F	M1	47, XX, +11, i(17q)	2.5	70	-	-	G	G

WBC: white blood cell. BM: bone marrow. FLT3-ITD: *FLT3* Internal tandem duplication. NA: not available. A: amplification of *MLL* gene observed by FISH analysis. G: germinal. ND: not done. M: mutated. R: rearrangement of *MLL* gene confirmed either by Southern Blot or FISH analyses. PTD: Partial tandem duplication detected by RT-PCR.

Microarray study: sample preparation for GeneChip Hibridization

Total RNA was extracted with Trizol (Invitrogen,Inc, Carlasbad,CA) reagent using the protocol provided by the manufacturer. The integrity of total RNA was confirmed in each case using the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

The sample preparation and processing procedure was performed at the IDIBAPS Genomic Unit following the protocols described in the Affymetrixs GeneChip Expression Analysis Manual (Affymetrix Inc., Santa Clara, CA). Briefly, five micrograms of total RNA was reverse transcribed into first strand cDNA using Superscript RT (Gibco Life Technology, NY), in the presence of T7-(T)24-oligo-dT primer containing a T7 RNA polymerase promoter and dNTPS. The second cDNA synthesis was performed using E. Coli DNA polymerase I. The ds-cDNA was cleaned using the Clean-up columns (Qiagen) and used it in an in vitro transcription reaction to generate cRNA containing biotinylated UTP and CTP using the High Yield Kit (Enzo diagnostics inc, Farmingdale NY). Biotinylated in vitro transcription products (cRNA) were purified using Clean-Up columns. The quality and length of the cRNA was tested using the Agilent 2100 Bioanalyzer. Fifteen μg of cRNA were fragmented by heat and ion-mediated hydrolysis at 94ºC for 35 minutes in a fragmentation buffer containing 40 mM of Tris acetate, pH 8.1, 100mM of potassium acetate, and 30mM magnesium acetate. The fragmented cRNA was used to prepare 300 ul of hybridization solution, containing sonicated herring sperm DNA 0.1 mg/ml, acetylated BSA 0.5 mg/ml, MES sodium salt 75 mM, MES free acid 27.5 mM, 0.005% Triton X-100 and internal controls for hybridization efficiency, including four bacterial and phage cRNA controls (1.5 pM BioB, 5 pM BioC, 25 pM BioD and 100 pM Cre) and control oligonucleotide (0.05 ug/ul oligo B2). Before hybridization, we heated the hybridization solution containing the fragmented cRNA to 95ºC for 5 min and then to 45ºC for 5 min before loading onto the Affymetrix HU133A probe array cartridge. The probe array was incubated for 16 hours at

45ºC with constant rotation at 60 rpm in an Affymetrix Gene Chip Oven 320. Subsequent washing and staining of the arrays was carried out using the Affymetrix Fluidics Station 450 following the manufacture instructions.

Table C. List of the 46 genes studied by real-time RT-PCR using low-density arrays.

	Gene Symbol	*Gene Title*
1	ADAMTS2	a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif, 2
2	AKR7A2	aldo-keto reductase family 7, member A2 (aflatoxin aldehyde reductase)
3	APBA2	amyloid beta (A4) precursor protein-binding, family A, member 2 (X11-like)
4	C20orf103	chromosome 20 open reading frame 103
5	CCND2	cyclin D2
6	CD44	CD44 antigen (homing function and Indian blood group system)
7	CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha
8	CHD3	chromodomain helicase DNA binding protein 3
9	CREBBP	CREB binding protein (Rubinstein-Taybi syndrome)
10	DAP	death-associated protein
11	DDB2	damage-specific DNA binding protein 2, 48kDa
12	EVI1	ecotropic viral integration site 1
13	FLT3	fms-related tyrosine kinase 3
14	GGA2	golgi associated, gamma adaptin ear containing, ARF binding protein 2
15	HIST1H2AG	histone 1, H2ag
16	HOXA3	homeo box A3
17	HOXA4	homeo box A4
18	HOXA7	homeo box A7
19	HOXA9	homeo box A9
20	HOXB3	homeo box B3
21	HOXB5	homeo box B5
22	HOXB6	homeo box B6
23	HOXB9	homeo box B9
24	ICSBP1	interferon consensus sequence binding protein 1
25	IRAK1	interleukin-1 receptor-associated kinase 1
26	ITGA7	integrin, alpha 7
27	LGALS3	lectin, galactoside-binding, soluble, 3 (galectin 3)
28	LMO2	LIM domain only 2 (rhombotin-like 1)
29	MEIS1	Meis1, myeloid ecotropic viral integration site 1 homolog (mouse)
30	MYST3	MYST histone acetyltransferase (monocytic leukemia) 3
31	OPTN	optineurin
32	PBX3	pre-B-cell leukemia transcription factor 3
33	PENK	proenkephalin
34	PNKP	polynucleotide kinase 3'-phosphatase
35	PPARG	peroxisome proliferative activated receptor, gamma
36	PRL	prolactin
37	RAD21	RAD21 homolog (S. pombe)
38	RET	ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)
39	RUNX1	runt-related transcription factor 1 (acute myeloid leukemia 1; aml1 oncogene)
40	S100A11	S100 calcium binding protein A11 (calgizzarin)
41	SATB1	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)
42	STAT3	signal transducer and activator of transcription 3 (acute-phase response factor)
43	STAT5A	signal transducer and activator of transcription 5A
44	STAT5B	signal transducer and activator of transcription 5B
45	SURF1	surfeit 1
46	WT1	Wilms tumor 1

Table D. List of the 63 genes distinctly overexpressed and 60 genes underexpressed in *MYST3-CREBBP* AML cases in the high-density array study (ratio of mean expression ≥ than 2 times the observed in each of the other groups).

Genes overexpressed		
	Gene Symbol	***Gene Title***
1	AAK1	AP2 associated kinase 1
2	ADCY9	adenylate cyclase 9
3	AGT	angiotensinogen (serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 8)
4	AP1S2	adaptor-related protein complex 1, sigma 2 subunit
5	APBA3	amyloid beta (A4) precursor protein-binding, family A, member 3 (X11-like 2)
6	ARTN	Artemin
7	ASL	argininosuccinate lyase
8	C20orf103	chromosome 20 open reading frame 103
9	C7orf32	chromosome 7 open reading frame 32
10	CD4	CD4 antigen (p55)
11	CPNE6	copine VI (neuronal)
12	CTSH	cathepsin H
13	DAP	death-associated protein
14	DDB2	damage-specific DNA binding protein 2, 48kDa
15	FBXL8	F-box and leucine-rich repeat protein 8
16	FLJ10815	amino acid transporter
17	FLJ20255	hypothetical protein FLJ20255
18	FLJ22386	leucine zipper domain protein
19	GAGED2	G antigen, family D, 2
20	GALIG	galectin-3 internal gene
21	GGA2	golgi associated, gamma adaptin ear containing, ARF binding protein 2
22	GGT1	gamma-glutamyltransferase 1
23	GPR25	G protein-coupled receptor 25
24	GTF3C5	general transcription factor IIIC, polypeptide 5, 63kDa
25	GYG2	glycogenin 2
26	HNMT	histamine N-methyltransferase
27	HOXA10	homeo box A10
28	HSPC163	HSPC163 protein
29	IQCE	IQ motif containing E
30	IRAK1	interleukin-1 receptor-associated kinase 1
31	KIAA0523	KIAA0523 protein
32	LYPLA3	lysophospholipase 3 (lysosomal phospholipase A2)
33	MOCOS	molybdenum cofactor sulfurase
34	NICAL	NEDD9 interacting protein with calponin homology and LIM domains
35	NME3	non-metastatic cells 3, protein expressed in
36	PCDH12	protocadherin 12
37	PHF10	PHD finger protein 10
38	PHF7	PHD finger protein 7
39	PHYH	phytanoyl-CoA hydroxylase (Refsum disease)
40	PLAG1	pleiomorphic adenoma gene 1
41	PLXNA1	plexin A1
42	PLXNB2	plexin B2
43	PPARG	peroxisome proliferative activated receptor, gamma
44	PRL	Prolactin
45	PROS1	protein S (alpha)
46	PTPN6	protein tyrosine phosphatase, non-receptor type 6

47	RET	ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)
48	RTN2	reticulon 2
49	S100A11	S100 calcium binding protein A11 (calgizzarin)
50	SATB2	SATB family member 2
51	SMTN	Smoothelin
52	SNX7	sorting nexin 7
53	STK11	serine/threonine kinase 11 (Peutz-Jeghers syndrome)
54	SURF1	surfeit 1
55	TAX1BP3	Tax1 (human T-cell leukemia virus type I) binding protein 3
56	TCEB2	transcription elongation factor B (SIII), polypeptide 2 (18kDa, elongin B)
57	TCIRG1	T-cell, immune regulator 1, ATPase, H+ transporting, lysosomal V0 protein a isoform 3
58	TP53I3	tumor protein p53 inducible protein 3
59	TRHDE	thyrotropin-releasing hormone degrading ectoenzyme
60	USP32	ubiquitin specific protease 32
61	VARS2	valyl-tRNA synthetase 2
62	ZFR	zinc finger RNA binding protein
63		Similar to lymphocyte-specific protein 1

Genes underexpressed

	Gene Symbol	***Gene Title***
1	ABHD2	Abhydrolase domain containing 2
2	ARSB	arylsulfatase B
3	ATP6V1C1	ATPase, H+ transporting, lysosomal 42kDa, V1 subunit C, isoform 1
4	BHLHB2	basic helix-loop-helix domain containing, class B, 2
5	BIRC4	baculoviral IAP repeat-containing 4
6	C13orf11	chromosome 13 open reading frame 11
7	C17	cytokine-like protein C17
8	C1orf24	chromosome 1 open reading frame 24
9	C2orf3	chromosome 2 open reading frame 3
10	CCND2	cyclin D2
11	CGI-49	CGI-49 protein
12	CHPT1	choline phosphotransferase 1
13	CPA3	carboxypeptidase A3 (mast cell)
14	CSPG6	chondroitin sulfate proteoglycan 6 (bamacan)
15	CUGBP1	CUG triplet repeat, RNA binding protein 1
16	DHX9	DEAH (Asp-Glu-Ala-His) box polypeptide 9
17	DLG1	discs, large homolog 1 (Drosophila)
18	DNAJB6	DnaJ (Hsp40) homolog, subfamily B, member 6
19	EGFR	epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian)
20	ELMO1	engulfment and cell motility 1 (ced-12 homolog, C. elegans)
21	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1
22	EPHB4	EPH receptor B4
23	EPRS	glutamyl-prolyl-tRNA synthetase
24	F11R	F11 receptor
25	FSCN1	fascin homolog 1, actin-bundling protein (Strongylocentrotus purpuratus)
26	HAO1	hydroxyacid oxidase (glycolate oxidase) 1
27	HBP1	HMG-box transcription factor 1
28	HIVEP2	human immunodeficiency virus type I enhancer binding protein 2
29	HK2	hexokinase 2
30	HTATSF1	HIV TAT specific factor 1
31	ITM2A	integral membrane protein 2A

32	KIAA0853	KIAA0853
33	LMAN1	lectin, mannose-binding, 1
34	MAN1A1	mannosidase, alpha, class 1A, member 1
35	MFHAS1	malignant fibrous histiocytoma amplified sequence 1
36	NCK1	NCK adaptor protein 1
37	NET1	neuroepithelial cell transforming gene 1
38	N-PAC	cytokine-like nuclear factor n-pac
39	PDE4B	phosphodiesterase 4B, cAMP-specific (phosphodiesterase E4 dunce homolog, Drosophila)
40	PFTK1	PFTAIRE protein kinase 1
41	PIK3R1	phosphoinositide-3-kinase, regulatory subunit 1 (p85 alpha)
42	PRDM2	PR domain containing 2, with ZNF domain
43	RAB6A	RAB6A, member RAS oncogene family
44	RAB8A	RAB8A, member RAS oncogene family
45	RANBP2	RAN binding protein 2
46	RYK	RYK receptor-like tyrosine kinase
47	SLC12A7	solute carrier family 12 (potassium/chloride transporters), member 7
48	SOS2	son of sevenless homolog 2 (Drosophila)
49	SPTBN1	spectrin, beta, non-erythrocytic 1
50	SYPL	synaptophysin-like protein
51	TBL1X	transducin (beta)-like 1X-linked
52	TGIF	TGFB-induced factor (TALE family homeobox)
53	TM4SF2	transmembrane 4 superfamily member 2
54	TRA1	tumor rejection antigen (gp96) 1
55	TRAM1	translocation associated membrane protein 1
56	UBE4B	ubiquitination factor E4B (UFD2 homolog, yeast)
57	ZNF395	zinc finger protein 395
58		Similar to microtubule-associated proteins 1A/1B light chain 3
59		CDNA: FLJ22642 fis, clone HSI06970
60		MRNA; cDNA DKFZp564F212 (from clone DKFZp564F212)

Table E. List of the 61 genes overexpressed in *MYST3-CREBBP* samples in common with another AML category in the high-density array study.

	Gene symbol	*AML category*
1	SEC23B	Monocytic-lineage
2	CCND3	Monocytic-lineage
3	AKR7A2	Monocytic-lineage
4	SPOCK	Monocytic-lineage
5	RXRA	Monocytic-lineage
6	GALE	Monocytic-lineage
7	IGF2R	Monocytic-lineage
8	OGFR	Monocytic-lineage
9	SLC16A3	Monocytic-lineage
10	RPS6KA1	Monocytic-lineage
11	VPS16	Monocytic-lineage
12	STS	Monocytic-lineage
13	MRPL33	Monocytic-lineage
14	RTN2	Monocytic-lineage
15	HK3	Monocytic-lineage
16	CHD3	Monocytic-lineage
17	CBR1	Monocytic-lineage
18	TST	Monocytic-lineage
19	FRAT2	Monocytic-lineage
20	OGFR	Monocytic-lineage
21	CCL23	Monocytic-lineage
22	STX12	Monocytic-lineage
23	AK2	Monocytic-lineage
24	C10orf56	Monocytic-lineage
25	FLNA	Monocytic-lineage
26	AKR7A3	Monocytic-lineage
27	MGC4368	Monocytic-lineage
28	DHRS9	Monocytic-lineage
29	CXorf21	Monocytic-lineage
30	DKFZp566O084	Monocytic-lineage
31	PYCARD	Monocytic-lineage
32	MYO7A	Monocytic-lineage
33	GM2A	Monocytic-lineage
34	PTPRF	*PML-RARα*
35	LAMC1	*PML-RARα*
36	QSCN6	*PML-RARα*
37	MRPL40	*PML-RARα*
38	DEXI	*PML-RARα*
39	CLTCL1	*PML-RARα*
40	P2RY2	*PML-RARα*
41	SLC35A2	*PML-RARα*
42	EFEMP2	*PML-RARα*
43	NCR3	*PML-RARα*
44	MAZ	*PML-RARα*
45	MOCS1	*PML-RARα*
46	TADA3L	*PML-RARα*

47	CD8B1	*PML-RARα*
48	GPR48	*PML-RARα*
49	C14orf123	*PML-RARα*
50	FAM45A	*PML-RARα*
51	SLC35A2	*PML-RARα*
52	EFEMP2	*PML-RARα*
53	PRKCZ	AML-MD
54	HOXA9	AML-MD
55	LOC388152	AML-MD
56	PSPC1	AML-MD
57	CD163	*CBFB-MYH11*
58	FLNB	*CBFB-MYH11*
59	NPTX2	*CBFB-MYH11*
60	SNRPN	*RUNX1-RUNX1T1*
61	ROBO1	*RUNX1-RUNX1T1*

Table F. List of the 87 genes underexpressed in *MYST3-CREBBP* in common with other AML category in the high-density array study.

	Gene Symbol	*Gene Title*
1	ADAM28	a disintegrin and metalloproteinase domain 28
2	ALCAM	Activated leukocyte cell adhesion molecule
3	APBB1IP	amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein
4	AREG	amphiregulin (schwannoma-derived growth factor)
5	ARNTL2	aryl hydrocarbon receptor nuclear translocator-like 2
6	BCAT1	branched chain aminotransferase 1, cytosolic
7	C6orf106	chromosome 6 open reading frame 106
8	CASP8	caspase 8, apoptosis-related cysteine protease
9	CCND2	cyclin D2
10	CD24	CD24 antigen (small cell lung carcinoma cluster 4 antigen)
11	CD74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)
12	CD84	CD84 antigen (leukocyte antigen)
13	CD86	CD86 antigen (CD28 antigen ligand 2, B7-2 antigen)
14	CDC2L2	cell division cycle 2-like 2
15	CHERP	calcium homeostasis endoplasmic reticulum protein
16	CPNE3	copine III
17	CSF3R	colony stimulating factor 3 receptor (granulocyte)
18	CTSW	cathepsin W (lymphopain)
19	DACH1	dachshund homolog 1 (Drosophila)
20	DCL-1	type I transmembrane C-type lectin receptor DCL-1
21	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1
22	EPB41L2	erythrocyte membrane protein band 4.1-like 2
23	ERO1L	ERO1-like (S. cerevisiae)
24	FAM46C	family with sequence similarity 46, member C
25	FBXO38	F-box protein 38
26	FBXW11	F-box and WD-40 domain protein 11
27	FLJ10074	hypothetical protein FLJ10074
28	FLJ14054	hypothetical protein FLJ14054
29	FUT7	fucosyltransferase 7 (alpha (1,3) fucosyltransferase)
30	GALNT1	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1 (GalNAc-T1)
31	GALNT2	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 2 (GalNAc-T2)
32	HLA-DMA	major histocompatibility complex, class II, DM alpha
33	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1
34	HMGA1	high mobility group AT-hook 1
35	IDS	iduronate 2-sulfatase (Hunter syndrome)
36	IL1RL1	interleukin 1 receptor-like 1
37	IL5RA	interleukin 5 receptor, alpha
38	IL6ST	interleukin 6 signal transducer (gp130, oncostatin M receptor)
39	IVNS1ABP	influenza virus NS1A binding protein
40	JUP	junction plakoglobin
41	KIAA0101	KIAA0101
42	KIAA0794	KIAA0794 protein
43	KIAA1212	KIAA1212
44	KIAA1449	WD repeat endosomal protein

45	LHFPL2	lipoma HMGIC fusion partner-like 2
46	LIMS1	LIM and senescent cell antigen-like domains 1
47	LOC91752	similar to C630007C17Rik protein
48	MALT1	mucosa associated lymphoid tissue lymphoma translocation gene 1
49	MGC8902	hypothetical protein MGC8902
50	MLLT10	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10
51	MYO5C	myosin VC
52	NAALADL1	N-acetylated alpha-linked acidic dipeptidase-like 1
53	NCOA2	nuclear receptor coactivator 2
54	N-PAC	cytokine-like nuclear factor n-pac
55	PAIP1	poly(A) binding protein interacting protein 1
56	PER2	period homolog 2 (Drosophila)
57	PHF3	PHD finger protein 3
58	PHTF1	putative homeodomain transcription factor 1
59	PHTF2	putative homeodomain transcription factor 2
60	PIK3R1	phosphoinositide-3-kinase, regulatory subunit 1 (p85 alpha)
61	PJA2	praja 2, RING-H2 motif containing
62	PLCL2	phospholipase C-like 2
63	PPFIA1	protein tyrosine phosphatase, receptor type, f polypeptide (PTPRF), interacting protein (liprin), alpha 1
64	PSME4	proteasome (prosome, macropain) activator subunit 4
65	RB1	retinoblastoma 1 (including osteosarcoma)
66	RIN3	Ras and Rab interactor 3
67	RNF125	ring finger protein 125
68	RNF130	ring finger protein 130
69	ROD1	ROD1 regulator of differentiation 1 (S. pombe)
70	RYBP	RING1 and YY1 binding protein
71	SNX13	sorting nexin 13
72	SOS2	son of sevenless homolog 2 (Drosophila)
73	STAT3	signal transducer and activator of transcription 3 (acute-phase response factor)
74	STOM	stomatin
75	TGIF2	TGFB-induced factor 2 (TALE family homeobox)
76	TIPARP	TCDD-inducible poly(ADP-ribose) polymerase
77	TM7SF3	transmembrane 7 superfamily member 3
78	TOPORS	topoisomerase I binding, arginine/serine-rich
79	UBXD2	UBX domain containing 2
80	UGCG	UDP-glucose ceramide glucosyltransferase
81	USP10	ubiquitin specific protease 10
82	VAV3	vav 3 oncogene
83	VEGF	vascular endothelial growth factor
84	YAF2	YY1 associated factor 2
85	ZNF281	zinc finger protein 281
86	ZNFN1A1	zinc finger protein, subfamily 1A, 1 (Ikaros)
87		MRNA; cDNA DKFZp564O0862 (from clone DKFZp564O0862)

Table G. List of the 53 genes overexpressed using a *t*-test comparison with 0.001 significance level.

	Gene Symbol	*Gene Title*
1	ABCD1	ATP-binding cassette, sub-family D (ALD), member 1
2	ALDH9A1	aldehyde dehydrogenase 9 family, member A1
3	APEH	N-acylaminoacyl-peptide hydrolase
4	APRT	adenine phosphoribosyltransferase
5	ARPC3	actin related protein 2/3 complex, subunit 3, 21kDa
6	ARTN	Artemin
7	ATP5E	ATP synthase, H+ transporting, mitochondrial F1 complex, epsilon subunit
8	BAG3	BCL2-associated athanogene 3
9	C12orf5	chromosome 12 open reading frame 5
10	C20orf103	chromosome 20 open reading frame 103
11	C20orf27	chromosome 20 open reading frame 27
12	C6orf49	chromosome 6 open reading frame 49
13	CAPZA1	capping protein (actin filament) muscle Z-line, alpha 1
14	CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha
15	COASY	Coenzyme A synthase
16	COX5A	cytochrome c oxidase subunit Va
17	CTSH	cathepsin H
18	DIPA	hepatitis delta antigen-interacting protein A
19	EDEM1	ER degradation enhancer, mannosidase alpha-like 1
20	FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide
21	FLJ10925	hypothetical protein FLJ10925
22	FLJ20643	hypothetical protein FLJ20643
23	GMIP	GEM interacting protein
24	GPX1	glutathione peroxidase 1
25	HT007	uncharacterized hypothalamus protein HT007
26	LRRC41	leucine rich repeat containing 41
27	LSM6	LSM6 homolog, U6 small nuclear RNA associated (S. cerevisiae)
28	MGC2477	hypothetical protein MGC2477
29	MGC3248	dynactin 4
30	MICAL1	microtubule associated monoxygenase, calponin and LIM domain containing 1
31	MRPS34	mitochondrial ribosomal protein S34
32	NDUFB4	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 4, 15kDa
33	NRD1	nardilysin (N-arginine dibasic convertase)
34	OPTN	optineurin
35	P4HB	procollagen-proline, 2-oxoglutarate 4-dioxygenase (proline 4-hydroxylase), beta polypeptide (protein disulfide isomerase-associated 1)
36	PEX16	peroxisomal biogenesis factor 16
37	PEX19	peroxisomal biogenesis factor 19
38	POLR2G	polymerase (RNA) II (DNA directed) polypeptide G
39	POLR2I	polymerase (RNA) II (DNA directed) polypeptide I, 14.5kDa
40	RET	Ret proto-oncogene (multiple endocrine neoplasia and medullary thyroid carcinoma 1, Hirschsprung disease)
41	RNH1	ribonuclease/angiogenin inhibitor 1
42	S100A11	S100 calcium binding protein A11 (calgizzarin)
43	SATB1	special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA's)
44	SDAD1	SDA1 domain containing 1

45	SFRS9	splicing factor, arginine/serine-rich 9
46	SKP1A	S-phase kinase-associated protein 1A (p19A)
47	SUCLG2	succinate-CoA ligase, GDP-forming, beta subunit
48	TOMM20	translocase of outer mitochondrial membrane 20 homolog (yeast)
49	TRAPPC4	trafficking protein particle complex 4
50	UQCRH	ubiquinol-cytochrome c reductase hinge protein
51	VAMP3	vesicle-associated membrane protein 3 (cellubrevin)
52	VPS11	vacuolar protein sorting 11 (yeast)
53	ZNF146	zinc finger protein 146

Table H. List of the 184 genes underexpressed using a *t*-test comparison with 0.001 significance level.

	Gene Symbol	*Gene Title*
1	---	LOC440669
2	ABCC1	ATP-binding cassette, sub-family C (CFTR/MRP), member 1
3	ABHD3	abhydrolase domain containing 3
4	ACTR2	ARP2 actin-related protein 2 homolog (yeast)
5	ADA	adenosine deaminase
6	ADAM28	a disintegrin and metalloproteinase domain 28
7	ADAM8	a disintegrin and metalloproteinase domain 8
8	ADSS	adenylosuccinate synthase
9	AGPAT5	1-acylglycerol-3-phosphate O-acyltransferase 5 (lysophosphatidic acid acyltransferase, epsilon)
10	AKAP11	A kinase (PRKA) anchor protein 11
11	AMD1	adenosylmethionine decarboxylase 1
12	ANGPT1	angiopoietin 1
13	ANP32B	acidic (leucine-rich) nuclear phosphoprotein 32 family, member B
14	ANXA1	annexin A1
15	AREG	amphiregulin (schwannoma-derived growth factor)
16	ARPP-19	cyclic AMP phosphoprotein, 19 kD
17	ATAD2	ATPase family, AAA domain containing 2
18	ATP1B1	ATPase, Na+/K+ transporting, beta 1 polypeptide
19	BCLAF1	BCL2-associated transcription factor 1
20	BIK	BCL2-interacting killer (apoptosis-inducing)
21	BRP44	brain protein 44
22	C11orf32	chromosome 11 open reading frame 32
23	C1orf24	chromosome 1 open reading frame 24
24	C21orf96	chromosome 21 open reading frame 96
25	C3orf4	chromosome 3 open reading frame 4
26	CCND2	cyclin D2
27	CD244	CD244 natural killer cell receptor 2B4
28	CD84	CD84 antigen (leukocyte antigen)
29	CD86	CD86 antigen (CD28 antigen ligand 2, B7-2 antigen)
30	CDCA8	cell division cycle associated 8
31	CHPT1	choline phosphotransferase 1
32	CLC	Charcot-Leyden crystal protein
33	COMT	catechol-O-methyltransferase
34	COPA	coatomer protein complex, subunit alpha
35	CPD	carboxypeptidase D
36	CPNE3	copine III
37	CRLF3	cytokine receptor-like factor 3
38	CSF3R	colony stimulating factor 3 receptor (granulocyte)
39	CTBP2	C-terminal binding protein 2
40	DACH1	dachshund homolog 1 (Drosophila)
41	DAF	decay accelerating factor for complement (CD55, Cromer blood group system)
42	DDX3X	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked
43	DJ328E19.C1.1	hypothetical protein DJ328E19.C1.1
44	DUT	dUTP pyrophosphatase
45	EIF2S3	eukaryotic translation initiation factor 2, subunit 3 gamma, 52kDa
46	ELMO1	engulfment and cell motility 1 (ced-12 homolog, C. elegans)

47	ENTPD1	ectonucleoside triphosphate diphosphohydrolase 1
48	EPS15	epidermal growth factor receptor pathway substrate 15
49	ERG	v-ets erythroblastosis virus E26 oncogene like (avian)
50	ETHE1	ethylmalonic encephalopathy 1
51	EXT2	exostoses (multiple) 2
52	F11R	F11 receptor
53	FAM61A	family with sequence similarity 61, member A
54	FAS	Fas (TNF receptor superfamily, member 6)
55	FCER1A	Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide
56	FLJ20719 / AG1	hypothetical protein FLJ20719 / AG1 protein
57	FSCN1	fascin homolog 1, actin-bundling protein (Strongylocentrotus purpuratus)
58	GAB2	GRB2-associated binding protein 2
59	GALNT7	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7 (GalNAc-T7)
60	GLUL	glutamate-ammonia ligase (glutamine synthase)
61	GRB10	growth factor receptor-bound protein 10
62	HINT1	histidine triad nucleotide binding protein 1 /// histidine triad nucleotide binding protein 1
63	HIRA	HIR histone cell cycle regulation defective homolog A (S. cerevisiae)
64	HK2	hexokinase 2
65	HLA-DQA1	major histocompatibility complex, class II, DQ alpha 1
66	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1
67	HLA-DRB1	major histocompatibility complex, class II, DR beta 1
68	HYPB	huntingtin interacting protein B
69	IFNGR1	interferon gamma receptor 1
70	IGLL1	immunoglobulin lambda-like polypeptide 1
71	IQGAP1	IQ motif containing GTPase activating protein 1
72	ITGA6	integrin, alpha 6
73	ITM1	integral membrane protein 1
74	IVNS1ABP	influenza virus NS1A binding protein
75	JARID2	Jumonji, AT rich interactive domain 2
76	KIAA0101	KIAA0101
77	KIAA0247	KIAA0247
78	KIAA0433	KIAA0433 protein
79	KIAA0470	KIAA0470
80	KIAA1212	KIAA1212
81	KIFC1	kinesin family member C1
82	KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
83	LHFPL2	lipoma HMGIC fusion partner-like 2
84	LIMS1	LIM and senescent cell antigen-like domains 1
85	LITAF	lipopolysaccharide-induced TNF factor
86	LOC440434	hypothetical protein FLJ11822
87	LY75	lymphocyte antigen 75
88	MAN1A1	mannosidase, alpha, class 1A, member 1
89	MAN2A2	mannosidase, alpha, class 2A, member 2
90	MARCH6	membrane-associated ring finger (C3HC4) 6
91	MAX	MYC associated factor X
92	MCL1	myeloid cell leukemia sequence 1 (BCL2-related)
93	MCM4	MCM4 minichromosome maintenance deficient 4 (S. cerevisiae)
94	MEST	mesoderm specific transcript homolog (mouse)
95	MLF1IP	MLF1 interacting protein

96	MLLT10	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 10
97	MPHOSPH9	M-phase phosphoprotein 9
98	MSH6	mutS homolog 6 (E. coli)
99	MYO5C	myosin VC
100	NASP	nuclear autoantigenic sperm protein (histone-binding)
101	NCK1	NCK adaptor protein 1
102	NECAP1	adaptin-ear-binding coat-associated protein 1
103	NET1	neuroepithelial cell transforming gene 1
104	NKTR	natural killer-tumor recognition sequence
105	NUCKS1	nuclear casein kinase and cyclin-dependent kinase substrate 1
106	NUP153	nucleoporin 153kDa
107	OPA1	optic atrophy 1 (autosomal dominant)
108	PAIP1	poly(A) binding protein interacting protein 1
109	PDE4B	phosphodiesterase 4B, cAMP-specific (phosphodiesterase E4 dunce homolog, Drosophila)
110	PFTK1	PFTAIRE protein kinase 1
111	PIP5K1B	phosphatidylinositol-4-phosphate 5-kinase, type I, beta
112	PJA2	praja 2, RING-H2 motif containing
113	PLAUR	plasminogen activator, urokinase receptor
114	PLCB1	phospholipase C, beta 1 (phosphoinositide-specific)
115	PLXND1	plexin D1
116	POLB	polymerase (DNA directed), beta
117	PRG2	proteoglycan 2, bone marrow (natural killer cell activator, eosinophil granule major basic protein)
118	PRKAR1A	protein kinase, cAMP-dependent, regulatory, type I, alpha (tissue specific extinguisher 1)
119	PRKCBP1	protein kinase C binding protein 1
120	PSIP1	PC4 and SFRS1 interacting protein 1
121	PTPLA	protein tyrosine phosphatase-like (proline instead of catalytic arginine), member a
122	PTPN11	protein tyrosine phosphatase, non-receptor type 11 (Noonan syndrome 1)
123	PTTG1	pituitary tumor-transforming 1
124	PUM1	pumilio homolog 1 (Drosophila)
125	RAB11FIP1	RAB11 family interacting protein 1 (class I)
126	RAB4A	RAB4A, member RAS oncogene family
127	RAD23B	RAD23 homolog B (S. cerevisiae)
128	RANBP2	RAN binding protein 2
129	RBBP8	retinoblastoma binding protein 8
130	RBM16	RNA binding motif protein 16
131	RGC32	response gene to complement 32
132	RNPC2	RNA-binding region (RNP1, RRM) containing 2
133	ROD1	ROD1 regulator of differentiation 1 (S. pombe)
134	RPL31	ribosomal protein L31
135	RRM2	ribonucleotide reductase M2 polypeptide
136	RTF1	Rtf1, Paf1/RNA polymerase II complex component, homolog (S. cerevisiae)
137	RYBP	RING1 and YY1 binding protein
138	SAMSN1	SAM domain, SH3 domain and nuclear localisation signals, 1
139	SCC-112	SCC-112 protein
140	SCCPDH	saccharopine dehydrogenase (putative)
141	SELL	selectin L (lymphocyte adhesion molecule 1)
142	SEPT6 / N-	septin 6 / cytokine-like nuclear factor n-pac

	PAC	
143	SET	SET translocation (myeloid leukemia-associated)
144	SF3A1	splicing factor 3a, subunit 1, 120kDa
145	SFPQ	Splicing factor proline/glutamine rich (polypyrimidine tract binding protein associated)
146	SLC12A7	solute carrier family 12 (potassium/chloride transporters), member 7
147	SLC2A5	solute carrier family 2 (facilitated glucose/fructose transporter), member 5
148	SLC39A8	solute carrier family 39 (zinc transporter), member 8
149	SLK	STE20-like kinase (yeast)
150	SMARCA2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2
151	SMARCA3	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 3
152	SORL1	sortilin-related receptor, L(DLR class) A repeats-containing
153	SPTBN1	spectrin, beta, non-erythrocytic 1
154	STAT5A	signal transducer and activator of transcription 5A
155	STAT5B	signal transducer and activator of transcription 5B
156	STK17B	serine/threonine kinase 17b (apoptosis-inducing)
157	STK38L	serine/threonine kinase 38 like
158	TBL1X	transducin (beta)-like 1X-linked
159	TBL1XR1	transducin (beta)-like 1X-linked receptor 1
160	TGFBRAP1	Transforming growth factor, beta receptor associated protein 1
161	TGIF	TGFB-induced factor (TALE family homeobox)
162	TGOLN2	trans-golgi network protein 2
163	TIPARP	TCDD-inducible poly(ADP-ribose) polymerase
164	TM7SF3	transmembrane 7 superfamily member 3
165	TMCO3	transmembrane and coiled-coil domains 3
166	TNFAIP3	tumor necrosis factor, alpha-induced protein 3
167	TOP1	topoisomerase (DNA) I
168	TRA1	tumor rejection antigen (gp96) 1
169	TRAM1	translocation associated membrane protein 1
170	TSPAN3	tetraspanin 3
171	TTC3	tetratricopeptide repeat domain 3
172	TXNRD1	thioredoxin reductase 1
173	UBAP2L	ubiquitin associated protein 2-like
174	UBE2G1	ubiquitin-conjugating enzyme E2G 1 (UBC7 homolog, C. elegans)
175	UBXD2	UBX domain containing 2
176	USP10	ubiquitin specific protease 10
177	VEGF	vascular endothelial growth factor
178	YLPM1	YLP motif containing 1
179	ZBTB1	zinc finger and BTB domain containing 1
180	ZCCHC11	zinc finger, CCHC domain containing 11
181	ZDHHC17	zinc finger, DHHC-type containing 17
182	ZNF22	zinc finger protein 22 (KOX 15)
183	ZNF364	zinc finger protein 364
184	ZNF395	zinc finger protein 395

Figure A. Genes overexpressed (A1) and underexpressed (A2) in *MYST3-CREBBP* samples (mean expression ratio ≥ 2 to the other AML subgroups).

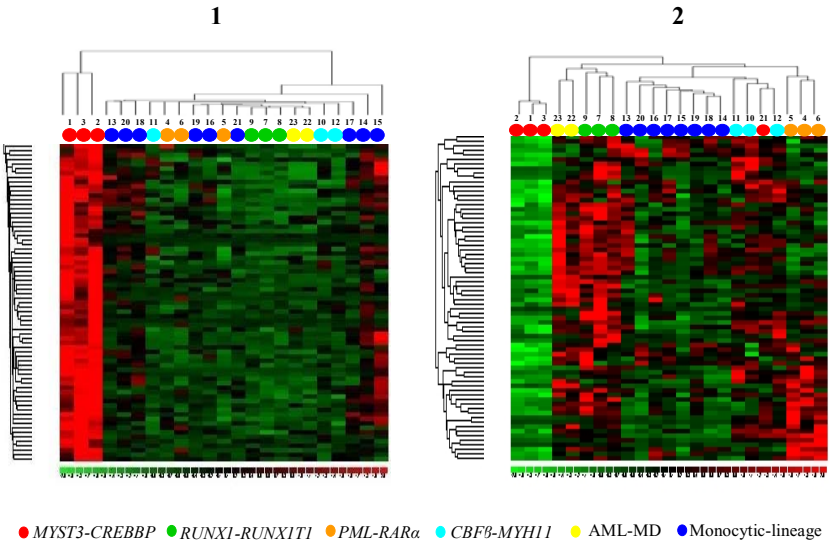

Figure B. Genes overexpressed (B1) and underexpressed (B2) in *MYST3-CREBBP* samples in common with one of the other AML categories.