Myeloid cell differentiation arrest by miR-125b-1 in myelodysplasic syndrome and acute myeloid leukemia with the t(2;11)(p21;q23) translocation

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Most chromosomal translocations in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) involve oncogenes that are either up-regulated or form part of new chimeric genes. The t(2;11)(p21;q23) translocation has been cloned in 19 cases of MDS and AML. In addition to this, we have shown that this translocation is associated with a strong up-regulation of miR-125b (from 6- to 90-fold). In vitro experiments revealed that miR-125b was able to interfere with primary human CD34$^+$ cell differentiation, and also inhibited terminal (monocytic and granulocytic) differentiation in HL60 and NB4 leukemic cell lines. Therefore, miR-125b up-regulation may represent a new mechanism of myeloid cell transformation, and myeloid neoplasms carrying the t(2;11) translocation define a new clinicopathological entity.

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MicroRNAs belong to a class of small noncoding RNAs of ~21 nt that control the expression of many genes (1, 2). MicroRNAs are preferentially transcribed by RNA polymerase II and can be derived from individual microRNA genes, introns of protein-coding genes, or polycistronic transcripts. They are first transcribed as primary microRNAs (pri-microRNAs) that

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correspond to capped and polyadenylated transcripts of ~1,000 nt. Pri-microRNAs are processed in the nucleus by the RNase Drosha into 70–80-nt, hairpin-shaped precursors, called pre-microRNAs (1, 2). They are then exported in the cytoplasm by exportin5, cleaved into mature microRNAs (21 nt) by the RNase III endonuclease Dicer, and incorporated in the RNA-induced silencing complex (1, 2). If the sequence alignment is perfect, the duplex microRNA–mRNA leads to degradation of the mRNA. If the alignment is incomplete, translation of the target mRNA is inhibited, but its stability is not affected (1).

Because microRNAs are involved in the regulation of many genes, they are suggested to control fundamental processes, such as differentiation, proliferation, and apoptosis. The disruption of their expression is now associated with several human diseases, including cancers (3–6). More than half of microRNAs are located at fragile sites and genomic regions implicated in cancer (7). A few reports have described chromosomal translocations that involve microRNAs (7, 8). The t(8;17)(q24;q22) translocation fuses miR-142 to the *c-MYC* oncogene and leads to overexpression of *c-MYC* in acute prolymphocytic leukemia (8, 9). Recent studies have shown that let-7 multiple target sites were located on the 3′ untranslated region of *HMGA2* (10). This is an example of suppression of an oncogene by a tumor-suppressive microRNA (10). One mechanism of oncogene activation is the occurrence of chromosomal translocations that eliminate the oncogene's 3′ untranslated region with the let-7 target sites. This impairs the repression by let-7 and leads to overexpression of HMGA2 (10). To the best of our knowledge, there is no paper mentioning chromosomal translocations leading to up-regulation of microRNAs in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML).

Chromosomal translocations are frequent in MDS and AML, and to date only rare abnormalities remain uncharacterized. Among them, the t(2;11)(p21;q23) chromosomal translocation is a rare event, although regularly reported (http://atlasgeneticsoncology.org) and specifically observed in patients with MDS and AML. In some cases, the translocation is isolated, suggesting that it could be the cause of deregulation of genes particularly relevant in myeloid cell transformation. Thus, we collected a series of 19 cases of AML or MDS carrying the t(2;11)(p21;q23) translocation. This translocation entailed an elevated expression of the microRNA miR-125b-1. In vitro transfections of miR-125b blocked the differentiation of leukemic cell lines upon chemical treatment. This property may account for the differentiation blockage observed in leukemic cells in vivo.

RESULTS AND DISCUSSION

The t(2;11)(p21;q23) chromosomal translocation is a clinicopathological entity

The t(2;11)(p21;q23) chromosomal translocation was found in 19 patients with AML (*n* = 10) or MDS (*n* = 9; Fig. 1 B;

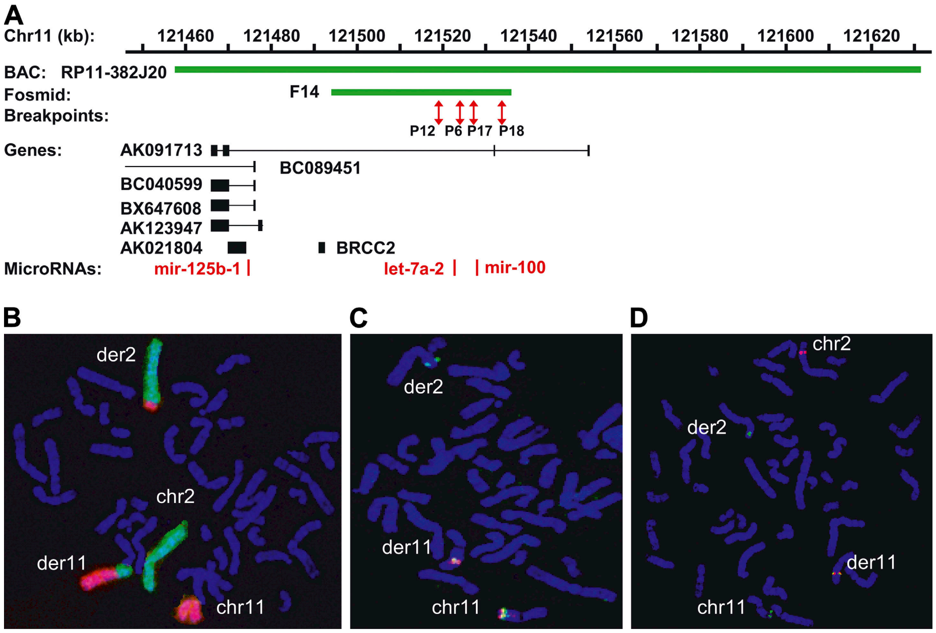

Figure 1. Identification of the breakpoints on chromosome 11. (A) Mapping of the BAC and fosmid probes on the breakpoint area and positions of the several mRNAs and microRNAs in this region. Vertical arrows show the exact position of the breakpoints for four patients obtained by LDI-PCR. (B) FISH showing chromosome painting of the t(2;11)(p21;q23) translocation in P1. Chromosome 2 is stained in green (biotin-streptavidin-FITC), and chromosome 11 is stained in red (digoxigenin-anti-dig-Rhodamine). (C) FISH with RP11-382J20 (green), as illustrated in A. The second BAC (red) is RP11-142I2 located at 11q23. Note that RP11-382J20 is split (P1). Of note, this BAC is split in 16 out of 19 patients (Table S1, available at http://www.jem.org/cgi/content/full/jem.20080285/DC1). (D) FISH with fosmid G248P85412D12 (green), as illustrated in A, and RP11-391M15 (red) mapping on chromosome 2p21 (P12). This fosmid is split in three patients (Table S1).

and Table S1, available at http://www.jem.org/cgi/content/full/jem.20080285/DC1). The median age of patients was 60 yr. The MDS were classified as refractory cytopenia with multilineage dysplasia, refractory cytopenia with multilineage dysplasia with ring sideroblasts, and refractory anemia with excess blasts. The AMLs were classified in AML with multilineage dysplasia in five patients or in AML evolving from myelodysplasia in three others. In two patients with AML, myelodysplastic signs could not be assessed. We can infer from these morphological data that AMLs with t(2;11) were acute phases of MDS in most patients.

The t(2;11) translocation was observed as a single abnormality in 5 patients and associated with other changes in 14. Because the t(2;11) was found to be the only change, and because it was never observed as a secondary change, we can infer that this translocation was the primary change in all karyotypes. In two patients, the normal chromosome 11 was lost and the derivative (11) was duplicated, which reinforces the critical role of chromosome 11 in the translocation. The secondary abnormalities associated with the t(2;11) translocation were the most frequent changes reported in MDS: deletion of the long arm of chromosome 5, del(5q), in eight patients and chromosome 7 abnormalities in four others, either complete monosomy 7 or partial deletion of the long arm, -7/del(7q). Both del(5q) and -7 were found in one patient. There was no correlation between morphological and cytogenetic features, because the isolated t(2;11) was found both in AML and in MDS and because the complexity of karyotypes was similar in both hemopathies. Furthermore, in all patients diagnosed either as AML or MDS, the t(2;11) translocation was present in major clones, whereas normal metaphases were either absent or present in minor cell populations, suggesting that the t(2;11) conferred a high proliferative advantage and was present in most dividing BM cells. Both morphological and cytogenetic data suggest that the t(2;11) translocation characterizes a single genetic entity with the phenotype either of MDS or AML.

The breakpoints on chromosomes 2 and 11 cloned by fluorescent in situ hybridization (FISH), long-distance PCR (LD-PCR), and long-distance inverse PCR (LDI-PCR) do not involve candidate genes

Material for FISH was available in all cases, whereas molecular techniques were possible in only six cases. *MLL*, located on 11q23, was not involved in the t(2;11) translocation in this type of pathology. By FISH, we showed that the breakpoint on chromosome 11 was located downstream from this gene (unpublished data). To determine the genes involved in this translocation, we progressively reduced the breakpoint regions on the two pathological chromosomes. The smallest interval was 10 megabases on chromosome 11. By FISH, using bacterial artificial chromosome (BAC) probes, we showed that the breakpoint occurred in the same chromosomal regions in all patients (*n* = 19; Fig. 1 A; and Table S2, available at http://www.jem.org/cgi/content/full/jem.20080285/DC1). The BAC probe RP11-382J20 mapping on chromosome 11 gave a split signal on both chromosomes, meaning that the breakpoint was within this area (on an interval of ~150 kb; Fig. 1 C and Table S2). To reduce this interval, we used fosmids (40 kb), which enabled us to shorten the breakpoint region on chromosome 11 to 40 kb (Fig. 1 D and Table S2). The same strategy was adopted for chromosome 2 and allowed us to define an interval of 300 kb (Table S2 and Fig. S1). Unexpectedly, no known genes were shown to map to these regions. By LDI-PCR and LD-PCR, we found the exact breakpoints for four patients (P6, P12, P17, and P18; Fig. 1 A, Table S3, Fig. S1, and Fig. S2).

MiR-125b-1 is overexpressed in patients with the t(2;11) translocation

We next tested by quantitative RT-PCR (RQ-PCR) the status of genes/expressed sequence tags located around the breakpoint region (*THADA*, *STS-1*, *BRCC2*, and *MTA3*) that could be deregulated in these pathologies, but we did not observe significant modifications of their expression (unpublished data). We focused our attention on the three microRNAs (miR-125b-1, let-7a-2, and miR-100) located near the breakpoint area on chromosome 11 (Fig. 1 A). By RQ-PCR, we compared their level of expression in patients with the t(2;11) translocation with six patients with MDS and five patients with AML without the t(2;11) translocation. We also compared the results with three BM samples from healthy individuals. Among the 19 patients, mRNA was available for 6 MDS patients (P11, P12, P16, P17, P18, and P19) and for 5 AML patients (P3, P7, P8, P9, and P10).

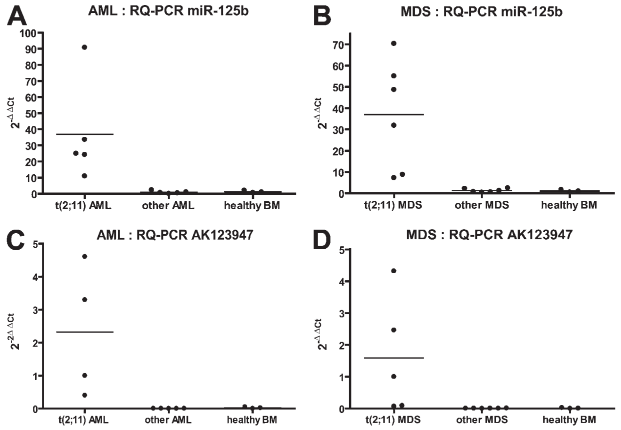

Figure 2. Overexpression of the mature miR-125b and one of its putative pri-microRNA (AK123947) in patients with the t(2;11)(p21;q23), as shown by RQ-PCR. (A) Up-regulation of the mature miR-125b in five AML patients with the translocation (P3, P7, P8, P9, and P10) compared with controls (five cases of AML without the t(2;11) and three BM samples from healthy individuals; $P < 0.05$). (B) The same results obtained in six MDS patients (P11, P12, P16, P17, P18, and P19). Controls are six MDS patients lacking the t(2;11) and three normal BM samples ($P < 0.05$). (C) The putative pri-microRNA AK123947 is also up-regulated in the AML patients (P3, P7, P8, and P9) compared with controls ($P < 0.05$). (D) The same results obtained in MDS patients (P11, P12, P16, P17, and P18; $P < 0.05$). Horizontal lines indicate mean values.

These experiments showed an elevated expression of the miR-125b (from 6- to 90-fold) in patients with the translocation compared with healthy individuals, or individuals with MDS and AML lacking the t(2;11)(p21;q23) translocation (Fig. 2, A and B). However, the production of the mature miR-125b depends on two loci located on chromosomes 11q23 and 21q21, and the RQ-PCR technique used did not allow discrimination between them. This prompted us to determine which could be the pri-microRNA of miR-125b-1. Fig. 1 A indicates that many mRNAs could be at the origin of miR-125b-1. RQ-PCR confirmed that mature miR-125b was transcribed from chromosome 11 (miR-125b-1 locus), because a strong expression of the mRNA AK123947 located on chromosome 11 was observed in patients with the translocation compared with control cases (Fig. 2, C and D).

MiR-125b overexpression significantly affects blast differentiation

The mechanisms accounting for miR-125b-1 up-regulation through the t(2;11)(p21;q23) translocation remain to be elucidated. However, such a high level of expression implies that miR-125b-1 could play a pivotal role in the pathogenesis of subsets of MDS and AML. Of note, in addition to the t(2;11) translocation, 10 cases also had a deletion of the 5q31 region. However, because there were some cases (*n* = 5) with isolated t(2;11), we suspected that this translocation was sufficient to interfere with the differentiation of myeloid cells.

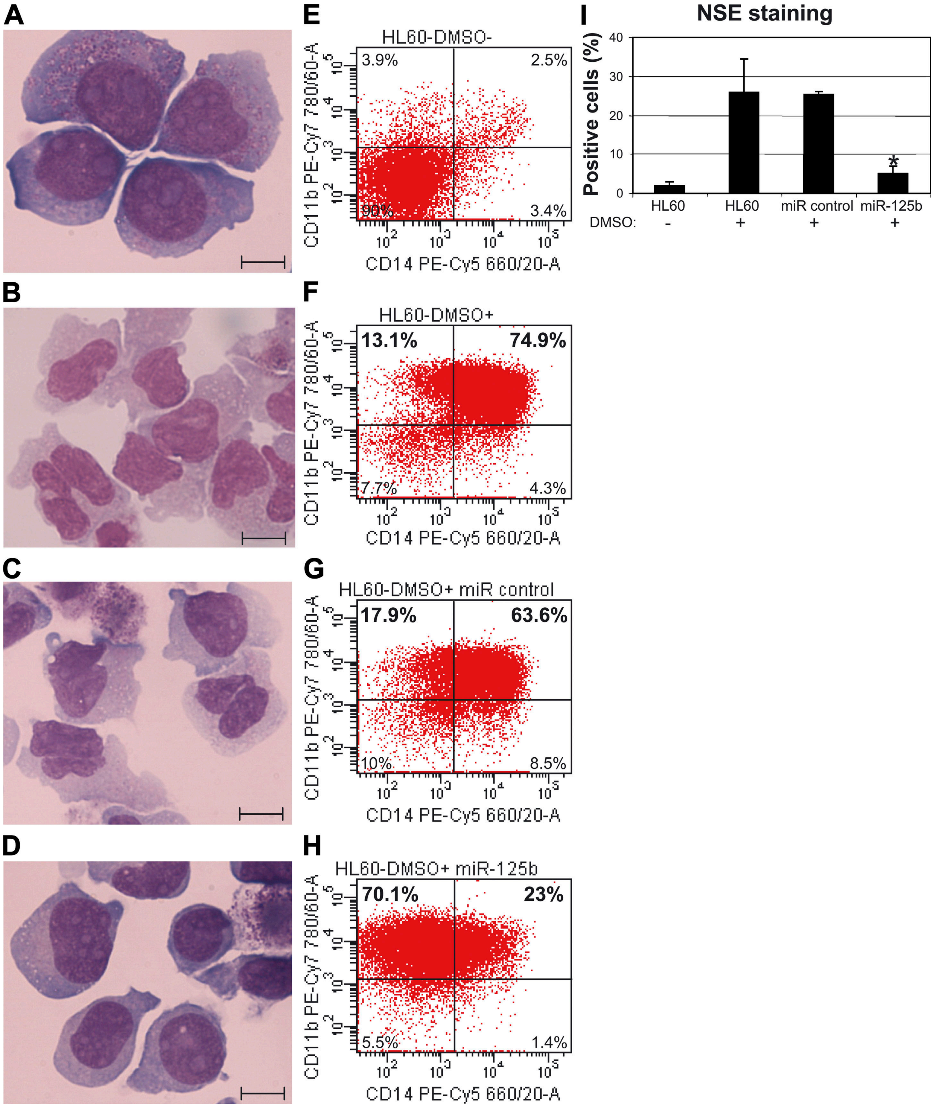

Figure 3. Transient transfection with miR-125b blocks the differentiation of HL60 cells induced by DMSO. (A–D) Changes in the morphology of May-Grunwald-Giemsa–stained cells (day 5): (A) HL60 cells electroporated with water (without DMSO), (B) HL60 cells electroporated with water (with DMSO), (C) HL60 cells electroporated with the microRNA negative control (with DMSO), and (D) HL60 cells electroporated with miR-125b (with DMSO). A representative experiment is shown. Bars, 5 µm. (E–H) Corresponding FACS experiments. A representative experiment is shown. (I) NSE staining. The data correspond to the mean from three independent experiments ($P < 0.05$).

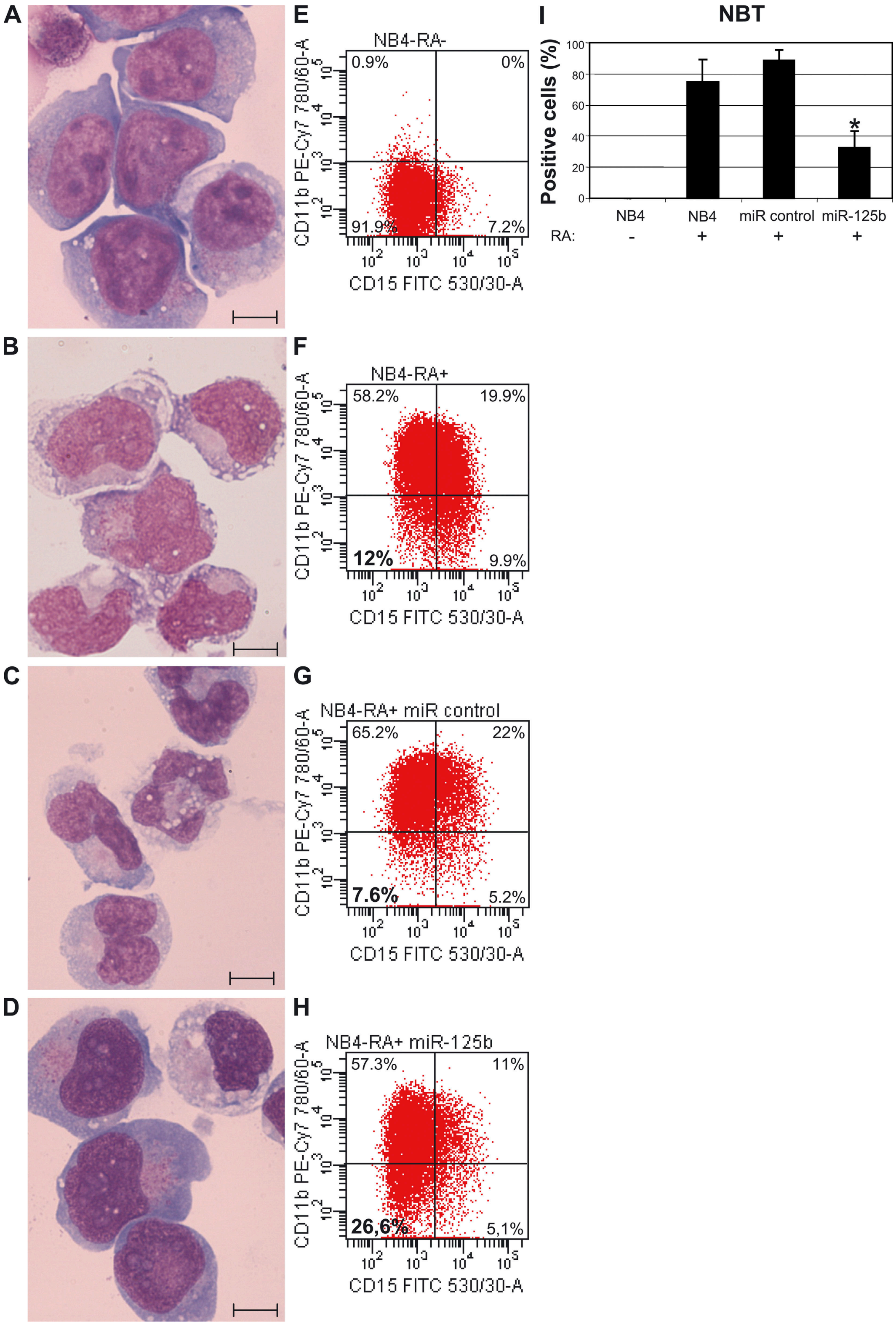

Figure 4. Transient transfection with miR-125b blocks the differentiation of NB4 cells induced by RA. (A–D) Changes in morphology of May-Grunwald-Giemsa–stained cells (day 5): (A) NB4 cells electroporated with water (without RA), (B) NB4 cells electroporated with water (with RA), (C) NB4 cells electroporated with the microRNA negative control (with RA), and (D) NB4 cells electroporated with miR-125b (with RA). A representative experiment is shown. Bars, 5 µm. (E–H) Corresponding FACS experiments. A representative experiment is shown. (I) NBT staining. The data correspond to the mean from three independent experiments ($P < 0.05$).

To address this question, we tested whether, in transfection experiments, miR-125b was able to block the differentiation of HL60 and NB4 leukemic cells upon chemical treatment. The experimental conditions allowed us to get a predominant maturation of HL60 into monocytic cells (after DMSO treatment), whereas NB4 cells treated with retinoic acid (RA) underwent characteristic granulocytic differentiation. We observed that in HL60 and NB4 cell lines, miR-125b was neither spontaneously up-regulated nor modulated during differentiation (unpublished data). After transient transfections, miR-125b significantly prevented the differentiation toward both lineages (Figs. 3 and 4). Regarding monocytic differentiation, the arrest of maturation was shown by both morphology (Fig. 3, A–D) and reduced expression of CD14 in HL60 cells (Fig. 3, E–H). These results were corroborated by those of the nonspecific esterase (NSE) staining (Fig. 3 I). Of note, it seemed that the blockage occurred between the expression of CD11b and CD14, i.e., in late stages of monocytic differentiation. As far as the granulocytic differentiation was concerned, the acquisition of CD11b was clearly affected by miR-125b transfection in NB4 cells upon RA treatment (Fig. 4, E–H). The maturation blockage was confirmed by morphological analysis (Fig. 4, A–D) and nitroblue tetrazolium (NBT) staining (Fig. 4 I).

MiR-125b overexpression affects CD34$^+$ primary blast differentiation

Several experimental conditions were used to transfect pools (10^6 cells) of human CD34$^+$ primary blasts (four experiments). The most significant effect was seen in transient transfections, as described for leukemic cells (see Materials and methods). Taking into account the criteria applied to myeloid cells in vitro, the results obtained with primary blasts revealed a blockage of differentiation that was particularly obvious with regard to morphological features. Roughly, we got half of differentiated cells upon miR-125b transfection compared with controls (Fig. 5, A–C). This was evaluated 8 d after induction of differentiation (upon GM-CSF treatment inducing both granulocytic and monocytic differentiation). Regarding the FACS results, in all experiments, there was a trend toward a delay in the acquisition of CD11b (variations of 5–10%; Fig. 5 D), but the results did not exactly fit with the morphological features seen for leukemic cells. This clearly suggests that the effect of miR-125b is greatly dependent on the stage and the pathway of differentiation of targeted cells. As seen in HL60 cells (with monocytic differentiation), miR-125b less significantly affected CD11b expression than in NB4 cells (upon granulocytic maturation).

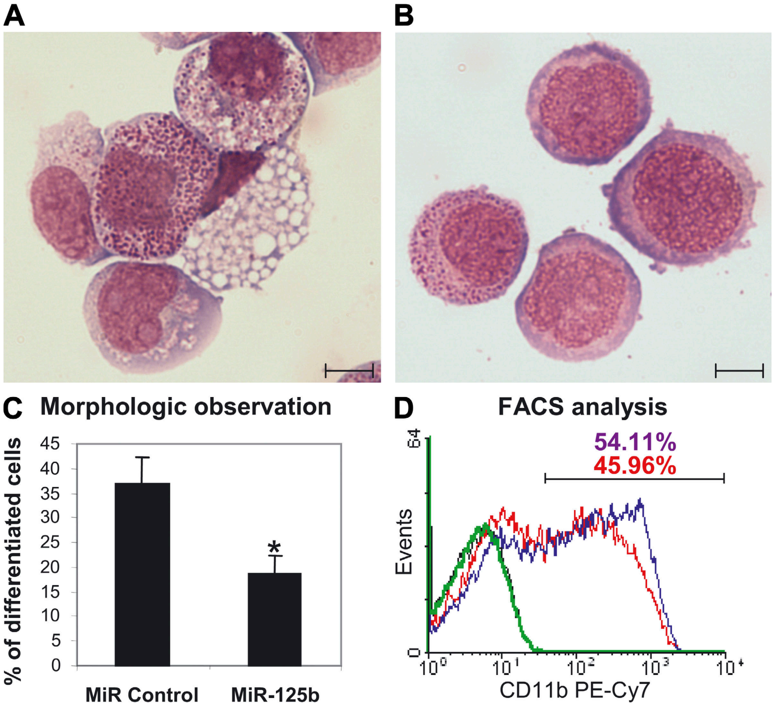

Figure 5. Transient transfection with miR-125b blocks the differentiation of CD34$^+$ primary cells. (A–C) Differentiation was evaluated by cell morphology with May-Grunwald-Giemsa–stained cytocentrifuge slides after 8 d of differentiation induced by IL-3 and GM-CSF treatment and transient transfection (at day 5) with the negative control (A) or miR-125b (B). Bars, 5 µm. The proportion of immature cells (B) is higher upon miR-125b transfection than in controls (A). (C) Graphic representation of morphological data. The number of differentiated cells includes myelocytes, metamyelocytes, neutrophils, monocytes, and macrophages compared with the number of undifferentiated cells (blasts, myeloblasts, and promyelocytes). The data correspond to the mean of four independent experiments ($P < 0.05$). (D) Corresponding CD11b expression by FACS analysis: primary blasts transfected with the negative control (blue, CD11b staining), and isotypic control [black] and primary blasts transfected with miR-125b (red, CD11b staining; green, isotypic control). A representative experiment is shown.

Among the candidate microRNAs located at fragile sites, in particular at 11q23, miR-125b-1 has been already reported, but its direct implication still remained hypothetical (7). A sporadic case of B acute lymphoblastic leukemia with a chromosomal translocation that rearranged miR-125b-1 with the IgH locus was reported, but no quantitative experiments were performed to determine whether this microRNA was deregulated (11). MiR125-b is critical in different processes of cell proliferation and differentiation, but most of the knowledge about its functions has been obtained from studies on its putative homologue *lin-4* in *Caenorhaditis elegans* (12). In particular, mutations in lin-4 are associated with developmental defects (10, 12, 13). The potential targets of miR-125b are listed in Table S4 (available at http://www.jem.org/cgi/content/full/jem.20080285/DC1). Because this microRNA has not been extensively studied, descriptions of target genes involved in hematopoietic differentiation are rare. We used a bioinformatic approach based on the combination of the four main softwares available to date (TargetScan, RNAhybrid, PicTar, and miRanda). Among the list of putative targets of miR-125b, *MLF2* (myelodysplasia/myeloid leukemia factor 2) and *MCL1* (myeloid cell leukemia 1) look promising.

The role of several microRNA sets in the molecular mechanisms that control myelomonocytic cell proliferation and differentiation has been documented (5, 14–18). In a very recent paper (15), the authors demonstrated a strong repression of the myelopoiesis regulator miR-223 by the AML1/ETO chimeric protein in AML. In the latter study, the molecular event was an epigenetic silencing of that microRNA. It is noteworthy that in normal conditions miR-223 promotes myeloid cell differentiation (15, 16). In the cases with the t(2;11) translocation, there is an up-regulation of miR-125b, which is directly linked to the chromosomal changes. In addition, miR-125b acts in an opposite direction by blocking the process of differentiation. This is a novel and so far unknown function for miR-125b.

In the study by Garzon et al., 60 untreated AML patients were tested for microRNA transcription profiles (19). A small subset of microRNAs correlated with survival, and some of them were associated with cytogenetic and molecular abnormalities (19). 8 microRNAs were up-regulated and 14 were down-regulated in cases with 11q23 balanced translocations. However, these profiles corresponded to cases with t(6;11) or t(9;11) translocations, usually involving the *MLL* gene. The fact that, in the latter study (19), miR-125b was not identified as a target strongly suggests that the up-regulation observed in our patients is specific to the t(2;11) translocation. This conclusion is reinforced by the very high level of expression (from 6- to 90-fold) of miR-125b-1 in tumor cells carrying the t(2;11) translocation. To strengthen the hypothesis that miR-125b is a key player in myeloid cell disorders, we retrieved cases with chromosomal abnormalities involving 21q21-22 (the region containing miR-125b-2). Interestingly, in one patient with AML, we observed that miR-125b-2 was strongly up-regulated (x450; Fig. S3, available at http://www.jem.org/cgi/content/full/jem.20080285/DC1).

We report herein the first chromosomal translocation up-regulating a microRNA in MDS and AML. The targeted microRNA miR-125b is capable of blocking the myelomonocytic differentiation of cell lines in vitro, a previously unknown function. Therefore, miR-125b up-regulation represents another mechanism of myeloid cell transformation that could be used as a diagnostic marker and therapeutic target in subsets of MDS and AML.

MATERIALS AND METHODS

Patient material. Cohorts studied included patients collected by the Laboratory of Hematology Cytogenetics, Centre Hospitalier Universitaire Purpan, and the Cytogenetic and Molecular Genetic Unit, Division of Hematology, IBiT Foundation, University of Perugia. French (reference: code Français de la santé publique Art.1.1211-2 alinea 2C) and Italian laws are similar and do not require consent for genetic analyses for dead or lost to follow-up patients. All patients enrolled (n = 19) were dead or lost to follow-up at the beginning of this study.

FISH. BAC and fosmid clones were obtained from the Welcome Trust Sanger Institute (http://www.sanger.ac.uk). DNA was labeled with biotin-16-dUTP or digoxigenin-11-dUTP (Roche), using a nick translation kit (GE Healthcare) according to the manufacturer's instructions.

LDI-PCR. DNA was digested with *PstI* or *ApaI* and purified by standard methods. The DNA was diluted to a concentration of 1 ng/µl and incubated at 14°C overnight in the presence of 1 U *T4* DNA ligase. The self-ligated circular DNA was used as a template in PCR experiments using the Advantage 2 PCR enzyme system (BD Biosciences) and primers listed in Table S3. Nucleotide sequencing of PCR products was performed with the ABI Prism Dye Terminator kit (PerkinElmer and Applied Biosystems).

RQ-PCR on microRNAs. RT was performed for each microRNA using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. Quantitative PCR was performed on an ABI7300 (Applied Biosystems) using TaqMan MicroRNA Assays (Applied Biosystems). The data presented correspond to the mean of $2^{-\Delta\Delta Ct}$ from three independent reactions, normalized to the *RNU43* reference gene.

RQ-PCR on mRNAs. cDNA was synthesized using M-MLV RT (Invitrogen) according to the manufacturer's instructions. Quantitative PCR was performed on an ABI7300 using qPCR MasterMix Plus for SYBR green (Eurogentec). AKf (5′-AGCTCAACGGAGGATTGTGCC-3′) and AKr (5′-TCAGTCTTTGTCCTTGCACTGG-3′) primers were used to amplify the *AK123947* cDNA. The data presented correspond to the mean of $2^{-\Delta\Delta Ct}$ from three independent reactions, normalized to the *MLN51* and *ACTIN* reference genes.

Cell lines and treatments. The HL60 and NB4 cell lines were cultured, respectively, in IMDM (Invitrogen) supplemented with 20% fetal bovine serum and in RPMI 1640 medium (Invitrogen) supplemented with 10% fetal bovine serum, L-glutamine, penicillin, and streptomycin.

Transient transfections were performed on 3×10^6 HL60 and NB4 cells with 22.5 µl of Pre-miR miRNA Precursor-miR-125b (50 µM), Pre-miR miRNA Precursor-Negative control #1 (50 µM; Applied Biosystems), or H_2O by electroporation at 950 µF and 280 V. After 24 h, HL60 and NB4 cells were differentiated into monocytes by treatment with 1.25% DMSO (Sigma-Aldrich) and into granulocytes with 1 µM RA (Sigma-Aldrich), respectively, for 5 d.

CD34$^+$ primary blasts (StemCell Technologies Inc.) were cultured in IMDM medium supplemented with 10% fetal bovine serum, penicillin, streptomycin, 50 ng/ml stem cell factor, 50 ng/ml FLT-3 Ligand, 10 ng/ml IL-3, and 50 ng/ml GM-CSF. Transient transfections were performed on 10^6 cells, after 5 d of differentiation, with 7.25 µl of Pre-miR miRNA Precursor-miR-125b

(50 μM) or Pre-miR miRNA Precursor-Negative control #1 (50 μM) by electroporation using the human CD34 cell nucleofector kit (Amaxa Biosystems).

Evaluation of myeloid differentiation. Monocytic differentiation was evaluated by cell morphology with May-Grunwald-Giemsa–stained cytocentrifuge slides, NSE staining as previously described (20), and expression of CD11b and CD14. Granulocytic differentiation was evaluated by cell morphology with May-Grunwald-Giemsa–stained cytocentrifuge slides, NBT (provided by C. Chomienne, St. Louis Hospital Paris, Paris, France) reduction activity as previously described (21), and expression of CD11b and CD15. $CD34^+$ primary blast differentiation was evaluated by cell morphology with May-Grunwald-Giemsa–stained cytocentrifuge slides and expression of CD11b (FACS). 300 cells were counted in each reaction (NSE, NBT, and morphology). For flow cytometry staining, the following anti–human monoclonal antibodies (provided by J.J. Fournié, Institut National de la Santé et de la Recherche Médicale, Toulouse, France) were used: CD11b-PE-Cy7 (1:100; BD Biosciences), CD14-PE-Cy5 (1:50; Beckman Coulter), and CD15-FITC (1:30; BD Biosciences). Flow cytometry analysis was performed using a flow cytometer (LSRII; BD Biosciences), and data were analyzed with FACSDiva software (BD Biosciences). A minimum of 10,000 events were collected for each sample.

Statistical analysis. For RQ-PCR experiments, the different groups were compared by a Kruskal-Wallis test. For NSE, NBT, and morphological analyses, the Mann-Whitney test was used. $P < 0.05$ was considered significant.

Online supplemental material. In Table S1, clinical data and complete karyotypes of the 19 patients are listed. In Table S2, results of FISH in all patients are shown. In Table S3, primers used in LDI-PCR experiments are listed. In Table S4, putative targets of miR-125b are listed. Fig. S1 describes breakpoint regions on chromosome 2 and shows FISH experiments on chromosome 2. Fig. S2 shows chromatograms of the breakpoint regions in four patients obtained by LDI-PCR or LD-PCR. Fig. S3 shows an up-regulation of miR-125b in a case of AML with amplification at 21q. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20080285/DC1.

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