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Massachusetts Institute of Technology

MicroRNA miR-125b causes leukemia

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MicroRNA miR-125b has been implicated in several kinds of leukemia. The chromosomal translocation t(2;11)(p21;q23) found in patients with myelodysplasia and acute myeloid leukemia leads to an overexpression of miR-125b of up to 90-fold normal. Moreover, miR-125b is also up-regulated in patients with B-cell acute lymphoblastic leukemia carrying the t(11;14)(q24;q32) translocation. To decipher the presumed oncogenic mechanism of miR-125b, we used transplantation experiments in mice. All mice transplanted with fetal liver cells ectopically expressing miR-125b showed an increase in white blood cell count, in particular in neutrophils and monocytes, associated with a macrocytic anemia. Among these mice, half died of B-cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, or a myeloproliferative neoplasm, suggesting an important role for miR-125b in early hematopoiesis. Furthermore, coexpression of miR-125b and the BCR-ABL fusion gene in transplanted cells accelerated the development of leukemia in mice, compared with control mice expressing only BCR-ABL, suggesting that miR-125b confers a proliferative advantage to the leukemic cells. Thus, we show that overexpression of miR-125b is sufficient both to shorten the latency of BCR-ABL–induced leukemia and to independently induce leukemia in a mouse model.

MicroRNAs are a family of small noncoding RNA (18–25 nucleotides) that act as crucial posttranscriptional regulators of genes involved in many fundamental process, including differentiation, proliferation, and apoptosis (1, 2).

Several microRNAs are involved in the differentiation process of various hematopoietic lineages. Indeed, miR-150 controls early B-lymphocyte differentiation (3, 4), miR-181 is a crucial modulator for T lymphocyte differentiation (5), microRNAs 17–5p, 20a, and 106a control monocytopoiesis (6), and miR-223 modulates the granulocytic lineage (7, 8). As microRNAs control the regulation of fundamental processes, their dysregulation has now clearly been linked to cancer and particularly to leukemia. As examples, overexpression of miR-155 has been found in many human leukemias and lymphomas, and mice transplanted with bone-marrow cells ectopically expressing miR-155 develop a myeloproliferative disorder (9). Deletion of the miR-15a/16–1 cluster has been detected in patients with chronic lymphocytic leukemia and causes leukemia in mice (10). The miR-17–92 cluster is frequently amplified in B-cell lymphomas in humans and its overexpression, along with c-myc, promotes development of B-cell lymphoma in a mouse model (11).

MicroRNA miR-125b is the ortholog of *lin-4* in *Caenorhaditis elegans*. As *lin-4* is implicated in several developmental processes (12), and because miR-125b negatively regulates many proteins in the p53 pathway (13–15), we hypothesized that deregulation of miR-125b expression would impair human and mouse hematopoiesis. MicroRNA-125b is transcribed from two loci located on chromosomes 11q23 (hsa-miR-125b-1) and 21q21 (hsa-miR-125b-2). MicroRNA-125b-1 is involved in several chromosomal translocations, such as t(2;11)(p21;q23) and t(11;14)(q24;q32), which leads to myelodysplasia and acute myeloid leukemia (AML) or B-cell acute lymphoid leukemia (B-ALL), respectively (16–18). In both cases, miR-125b overexpression is the only consistent abnormality found in these patients, suggesting that it is a main oncogenic event. Moreover, miR-125b-2 has been shown to be overexpressed in patients with trisomy 21/Down Syndrome associated with megakaryoblastic leukemia (19). Recently, miRNA profiling of lineage-negative, Sca^+, $cKit^+$ (LSK) cells revealed that miR-125b and its homolog miR-125a are highly expressed in hematopoietic stem cells. In these studies, miR-125b was found to regulate stem-cell pool size and to confer a competitive advantage to engrafting hematopoietic cells (20, 21).

In this study, we report the capacity of miR-125b to transform cells and induce leukemia affecting both myeloid and lymphoid lineages. We also show that miR-125b is able to accelerate the tumorigenicity of the BCR-ABL fusion protein, suggesting that miR-125b can also be involved in leukemia as a second event.

Results

MicroRNA-125b Overexpression in Murine Hematopoietic Cells Leads to an Increase in WBCs Associated with Macrocytic Anemia. To test if miR-125 overexpression is sufficient to induce leukemia in vivo, we used transplantation experiments in mice. To this end, we first developed a retroviral vector to induce the expression of mature miR-125b, and used the bicistronic retroviral vector XZ, which contains a RNA polymerase II promoter driving miRNA expression followed by an internal ribosome entry site (IRES) and GFP. Thus, GFP expression marks cells ectopically expressing miR-125b.

Lineage-negative hematopoietic fetal liver cells (C57BL/6 CD45.2) were infected with XZ-miR-125b or XZ alone and injected into lethally irradiated recipient mice (C57BL/6 CD45.1). Donor infected cells can be distinguished from other cells by flow cytometry analysis detecting the presence of the CD45.2 marker and GFP. The level of expression of miR-125b at 16 wk posttransplantation was elevated around 700-fold normal in the peripheral blood of two independent batches of mice transplanted with cells expressing miR-125b, as shown in Fig. 1*A*. We collected peripheral blood once a month, analyzed the complete blood count (CBC), and used flow cytometry to measure the different hematopoietic compartments (CD4 and CD8 staining for T cells, B220 staining for B cells, and CD11b and Gr1 staining for myeloid cells). We observed an increase in the percent of GFP^+ cells in the peripheral blood over time, suggesting that miR-125b overexpression confers a proliferative advantage to hematopoietic cells (Fig. 1*B*). Indeed, CBC results done at 16 wk posttransplant showed an increase in WBC in mice expressing miR-125b compared with control mice (Fig. 1*C*). In particular, there were statistically significant increases in neutrophils, monocytes, and lymphocytes (Fig. 1*C*). A representative flow cytometry plot shows the increase in the percentage of myeloid cells in miR-125b–expressing mice compared with control mice (Fig. 1*D*). The numbers of monocytic cells ($CD11b^+Gr1^-$) and neutrophils ($CD11b^+Gr1^+$) were systematically higher in mice transplanted with miR-125b–infected cells compared with the control. This increase in WBCs was also associated with a macrocytic anemia as illustrated by the decrease in RBC count, hemoglobin levels, and hematocrit, and the increase in

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The authors declare no conflict of interest.

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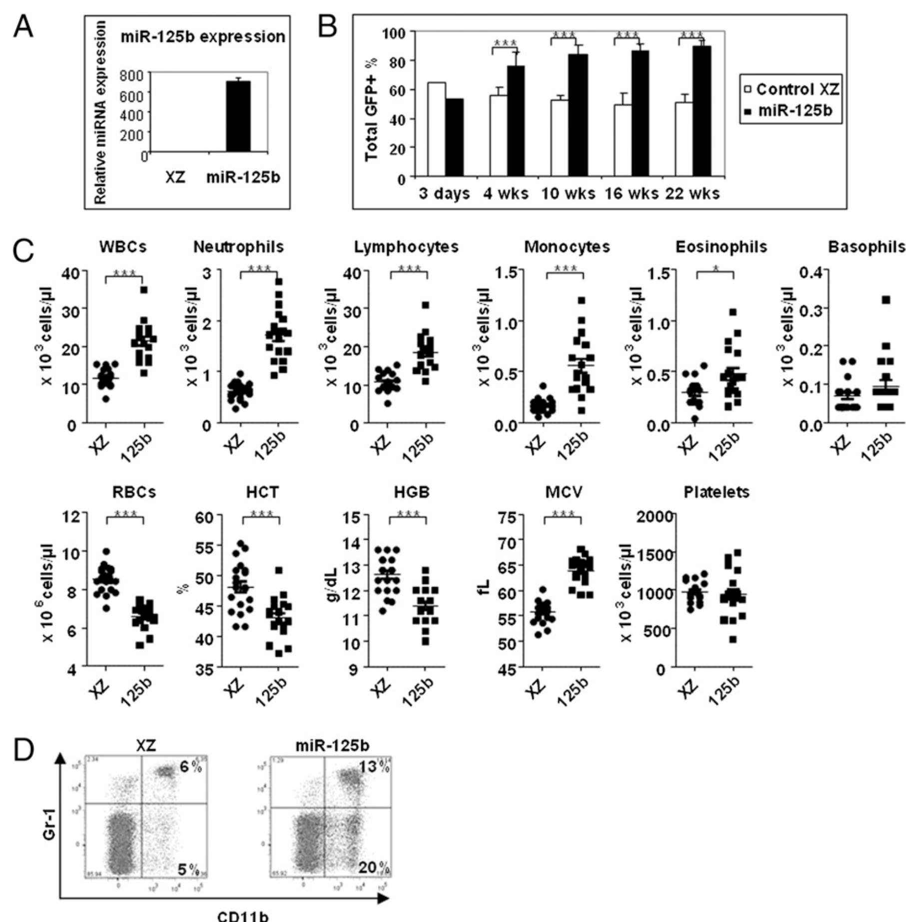

Fig. 1. MicroRNA-125b overexpression leads to an increase in WBCs associated with a macrocytic anemia in mice. C57BL/6 mice (CD45.1) were transplanted with lineage-negative hematopoietic fetal liver cells (CD45.2) that were infected with an empty retroviral vector (XZ) expressing GFP or infected with the same vector expressing GFP and miR-125b. Peripheral blood was harvested once a month for CBCs and flow cytometry analysis. (*A*) MicroRNA-125b expression level relative to controls in WBCs (after lysis of red cells) of mice from two different batches at 16 wk posttransplantation. (*B*) Percent of GFP^+ cells in the peripheral blood of mice transplanted with miR-125b or control XZ infected cells over time. The first time point corresponds to the percent of GFP^+ cells 3 d after the first infection in in vitro culture. *t* test: ****P* < 0.0001. (*C*) Whole-blood counts of mice transplanted with control (*n* = 20) or miR-125b infected cells (*n* = 18) showing numbers of WBC, the absolute numbers of neutrophils, lymphocytes, monocytes, eosinophils and basophils, RBCs, platelets, percent hematocrit, hemoglobin concentration, and MCV (mean volume of red blood cells) at 16 wk posttransplantation. Combined data from three independent experiments are shown. Mann–Whitney test: **P* < 0.05; ****P* < 0.0001. (*D*) Representative peripheral blood flow cytometry at 16 wk posttransplantation from mice transplanted with XZ control or miR-125b–infected cells. $Cd11b^+Gr1^+$ cells correspond to granulocytes; $Cd11b^+Gr1^-$ cells correspond to monocytes.

mean erythroid cell volume value (MCV) in mice transplanted with miR-125b–expressing cells (Fig. 1*C*). Of note, these data correspond to 16 wk posttransplantation. However, at that time two mice ectopically expressing miR-125b had already died, meaning that the hematopoietic abnormalities observed in Fig. 1 are slightly underestimated because two of the most profoundly affected mice were excluded because of death.

MicroRNA-125b Overexpression Causes Leukemia in Mice. Among the mice transplanted with miR-125b–overexpressing fetal liver cells, half of them succumbed to a hematologic malignancy within 12 to 29 wk posttransplantation (Fig. 2*A* and Table 1). We observed different phenotypes, including a myeloproliferative neoplasm (MPN), B-ALL, and T-ALL (Table 1). As shown in Fig. 2 *D* and *E*, Mouse 1 displayed a MPN with an excess of neutrophils in the peripheral blood, a red pulp expanded by neutrophils in the spleen, and a myeloid-predominant marrow (Fig. 2*E* and Fig. S1) compared with that of a control mouse (Fig. 2*C*). Granulocytes ($CD11b^+Gr1^+$) were the major population in the blood, bone marrow, and spleen (Fig. 2*D*).

Mice 2 and 10 exhibited a B-cell ALL characterized by lymphoblasts in the peripheral blood and the bone marrow, as shown by flow cytometry with the B220 marker and by histology (Fig. 2 *F* and *G*). Splenic architecture was also disrupted by the presence of lymphoblasts (Fig. 2*G* and Fig. S1).

Mice 3, 4, 6, 7, and 9 developed a T-ALL. As evidence, in the peripheral blood of mouse 4, 7% of the cells were $CD4^+CD8^+$ double-positive T-cell progenitors (Fig. 2*H*). Of note, this mouse also displayed monocytosis in the peripheral blood. Histology showed that the bone marrow and spleen of this mouse were packed with lymphoblasts, but flow cytometry demonstrated a $CD4^+CD8^+$ phenotype (Fig. 2 *H* and *I*, and Fig. S1).

Two other mice were found dead at 20 and 28 wk, respectively, preventing determination of the cause of death. Notably, there were no deaths in the control group.

Using WBCs from peripheral blood of six leukemic mice, we performed quantitative RT-PCR to evaluate the level of miR-125b in the different types of leukemia. As shown in Fig. 2*B*, the type of leukemia seems to be related to the level of expression of miR-125b, with a lower level in T-ALL and a higher level in myeloid neoplasm.

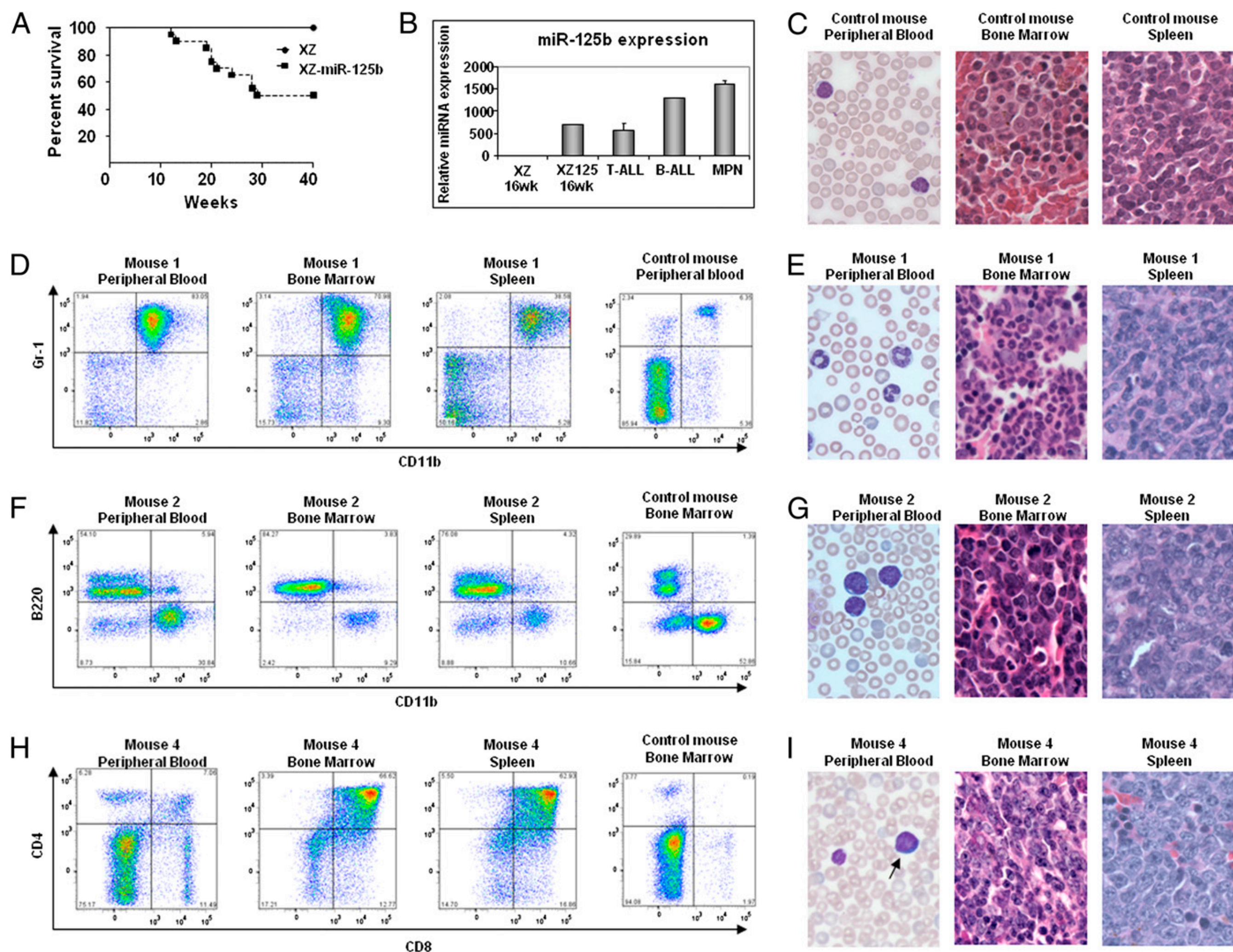

Fig. 2. MicroRNA-125b overexpression causes leukemia in mice. Of 20 mice transplanted with miR-125b–infected lineage-negative fetal liver cells in three different batches, 10 developed a fatal hematologic malignancy. Peripheral blood samples, bone marrow, and spleen single-cell suspensions were collected from moribund mice. Donor-derived cells (CD45.2) were stained by using antibodies against specific lineage markers to determine the proportion of the different lineages by flow cytometry analysis: myeloid cells (CD11b and Gr1), B cells (B220), and T cells (CD4 and CD8). Peripheral blood smears were stained with May Grunwald Giemsa (MGG). Five micrometer sections of paraffin-embedded bone marrow and spleen tissue were prepared on slides and stained with H&E. (*A*) Survival curve of mice transplanted with miR-125b or control vector-infected cells. (*B*) Quantitative RT-PCR on peripheral blood of leukemic mice with T-ALL (n = 3), B-ALL (n = 1), or MPN (n = 2) compared with healthy mice transplanted with miR-125b or control vector-infected cells at 16 wk post-transplantation. (*C*) Representative MGG staining of peripheral blood smear (100×) and H&E staining of bone marrow (100×) and spleen (100×) sections from a control mouse killed at 16 wk posttransplantation. (*D*) Representative flow cytometry from a mouse with a MPN (mouse 1) compared with peripheral blood staining of a control mouse. Peripheral blood, bone marrow, and spleen of mouse 1 are full of neutrophils ($CD11b^{+}Gr1^{+}$). (*E*) Corresponding MGG staining of peripheral blood smear (100×) and H&E staining of bone marrow (100×) and spleen (100×) sections showing an excess of myeloid cells. (*F*) Representative flow cytometry from a mouse with B-ALL (mouse 2) compared with bone-marrow staining of a control mouse. Note the presence of lymphoblasts ($B220^{+}$) in the peripheral blood, bone marrow, and spleen. (*G*) Corresponding MGG staining of peripheral blood smear (100×) and H&E staining of bone marrow (100×) and spleen (100×) sections. (*H*) Representative flow cytometry from a mouse with T-ALL (mouse 4) compared with bone-marrow staining of a control mice. Presence of $CD4^{+}CD8^{+}$ lymphoblasts in the peripheral blood, bone marrow, and spleen. (*I*) Corresponding blood smear (100×) showing a monocyte and a lymphoblast (arrow). Bone marrow (100×) and spleen (100×) sections with excess of lymphoblasts.

MicroRNA-125b Overexpression Accelerates the Development of BCR-ABL–Induced Leukemia in Mice. Clearly, miR-125b is an oncogene, in that its ectopic expression in hematopoietic stem/progenitor cells induces multiple types of leukemia after 12 to 29 wk post-transplantation. To determine whether miR-125b overexpression could also be a secondary event able to increase oncogenicity of another oncogene, we used an established leukemic mouse model.

Royer-Pokora et al. reported a case of human chronic myelogenous leukemia with a t(2;11)(p21;q23) translocation associated with the BCR-ABL translocation (22). The expression of miR-125b was not assessed, but the described breakpoints are the same as those in the t(2;11)(p21;q23) translocation found in AML and myelodysplastic syndrome, suggesting a possible cooperation between the BCR-ABL fusion protein and overexpression of miR-125b. To check if miR-125b overexpression is able to affect the oncogenicity of BCR-ABL, we coinfected 5-fluorouracil (5-FU)–treated bone-marrow cells with a retrovirus encoding p210 BCR-ABL, together with the XZ-miR-125b–overexpressing miR-125b or the control vector XZ. We then transplanted these cells into lethally irradiated BALB/c recipient mice. We demonstrated the presence of the ectopic BCR-ABL by RT-PCR in the bone marrow of BCR-ABL control and BCR-ABL–miR-125b

Table 1. Summary of hematopoietic disease development in miR-125b reconstituted mice

Mice	Pathology	Death	WBC	RBC	HGB	HCT	MCV	PLT
1	MPN	12 wk	84.2	4.2	6.4	29.2	70.1	68
2	B-ALL	13 wk	40.12	6.04	10.4	38.4	64.1	288
3	T-ALL	19 wk	8.88	6.68	10.4	44.8	67	780
4	T-ALL	20 wk	93.08	6.6	12	50	76	288
5	Unidentified	20 wk						
6	T-ALL	21 wk	58.64	2.88	12.8	19.2	67.1	100
7	T-ALL	24 wk	16.48	3.28	14	55.2	73	144
8	Unidentified	28 wk						
9	T-ALL	28 wk	150.8	2.96	12.4	19.2	63.6	116
10	B-ALL	29 wk	12.52	5.8	10.4	38	65.8	596
Control mice			11.9 ± 2.5	8.5 ± 0.6	12.6 ± 0.7	48 ± 3.7	56 ± 2.2	1,004 ± 116

Ten out of 20 mice died after transplantation with miR-125b–infected fetal liver cells between 12 and 29 wk posttransplantation. Moribund mice were killed and peripheral blood was analyzed by CBC. "Unidentified" corresponds to mice found dead. Death indicates the number of weeks after transplantation. Control mice correspond to the average of CBC values at 16 wk posttransplantation of mice transplanted with XZ infected cells. B-ALL, B-cell acute lymphoblastic leukemia; HCT, hematocrit (%); HGB, hemoglobin (g/dL); MCV, mean cell volume (fL); MPN, myeloproliferative neoplasm; PLT, platelets (×10^3 cells per μL); RBC, red blood cells (×10^6 cells per μL); T-ALL, T cell acute lymphoblastic leukemia; WBC, white blood cells (×10^3 cells per μL).

mice (Fig. 3*A*). Of note, the white cells from mice transplanted with miR-125b-BCR-ABL–infected cells overexpressed miR-125b 500-fold compared with BCR-ABL mice (Fig. 3*B*). All mice from three independent experiments with a total of 20 mice per group developed a fatal MPN or B-ALL (Fig. 3*C*). Among the mice transplanted with miR-125b plus BCR-ABL–infected cells, 50% died of B-ALL, 42% with MPN, and 8% of mixed (myeloid and lymphoid) leukemia. In the control group (BCR-ABL only), the proportion of B-ALL observed was 40%, 53% of MPN, and 7% of mixed leukemia. However, mice transplanted with BCR-ABL-miR-125b–infected cells had a median survival of 21 d compared with 35 d in the control group (Fig. 3*C*). A log-rank test comparing survival curves of mice transplanted with cells expressing or not miR-125b gave a *P* value <0.0001, suggesting that the difference of disease latency between the two groups of mice is statistically significant. Thus, miR-125b accelerates the oncogenicity of BCR-ABL in this transplanted mouse model.

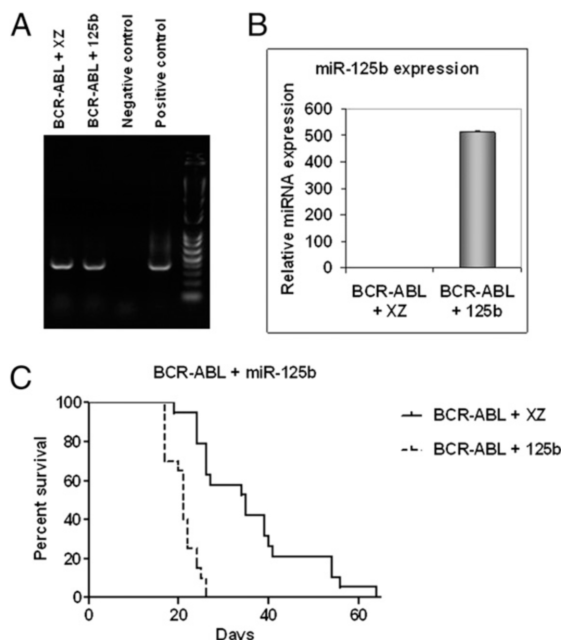

Fig. 3. MicroRNA-125b overexpression decreases the latency of BCR-ABL–induced leukemia. BALB/c mice were transplanted with 5-FU–treated bone-marrow cells that were infected with retroviral vectors expressing BCR-ABL and an empty vector XZ or infected with retroviral vectors expressing BCR-ABL and miR-125b. (*A*) Example of BCR-ABL RT-PCR on bone-marrow cells from a mouse transplanted with BCR-ABL–infected cells or miR-125b plus BCR-ABL–infected cells. PCR was processed with a negative control corresponding to cDNA from an untransplanted mouse and a positive control corresponding to the BCR-ABL construct. (*C*) Survival curve. Mice transplanted with BCR-ABL and miR-125b–infected bone-marrow cells (n = 20) have a median survival of 21 d. Mice transplanted with BCR-ABL and XZ-infected bone-marrow cells (n = 19) have a median survival of 35 d. Combined data from three independent experiments are shown. Log-rank test gives a *P* value <0.0001.

Discussion

We report here that miR-125b overexpression promotes malignant transformation of different hematopoietic lineages in mice. Indeed, B-ALL, T-ALL, and a MPN were observed, suggesting a role of miR-125b in the differentiation process of lymphoid and myeloid lineages. MiR-125b has already been shown to arrest myeloid differentiation in human cell lines (16). Transient transfection of a miR-125b mimic blocks in vitro granulocytic and monocytic differentiation of NB4 and HL-60 cells, respectively (16). Klusmann et al. showed that miR-125b overexpression blocks myeloid differentiation of primary progenitors (19). In our hands, transplantation of mice with hematopoietic progenitors overexpressing miR-125b leads to a MPN without any signs of myeloid differentiation arrest.

Recently, O'Connell et al. showed that transplantation of mice with bone-marrow cells overexpressing miR-125b causes a MPN that progresses to AML in transplanted mice within 15 to 23 wk posttransplantation (21). The only pathology reported in that study was AML and most of the mice described succumbed to leukemia. In our hands, only 50% of mice transplanted with hematopoietic progenitors overexpressing miR-125b died between 12 and 29 wk posttransplantation, and they exhibited different leukemic phenotypes. The differences observed could be explained by the level of expression of miR-125b in the mice. Indeed, we observed by quantitative RT-PCR that the level of expression of miR-125b in the leukemic mice is related to the type of leukemia. Moreover, O'Connell et al. used three different miR-125b constructs, which lead to different levels of expression in infected cultured K562 cells. The authors used these different vectors in transplanted mice and concluded that the MPN caused by miR-125b progressed into AML in a dose-dependent manner (21). In humans, the level of overexpression of miR-125b in

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patients carrying the translocation t(2;11)(p21;q23) in AML and myelodysplasia is 6- to 90-fold higher than in other AML cases or in healthy patients (16). In patients with B-ALL, the translocation t(11;14)(q24;q32) leads to an overexpression of miR-125b 30- to 600-fold compared with B-ALL cases without the translocation (17). Thus, the human data suggests that overexpression of miR-125b can lead to multiple types of leukemia, as we observed in our mouse transplant experiments. Regarding the different leukemic phenotypes induced by miR-125b overexpression, it will be of interest to identify if the transformation occurs in hematopoietic stem cells or in downstream progenitors.

MicroRNA-125b and its homolog miR-125a have recently been shown to be highly expressed in hematopoietic stem cells and when ectopically overexpressed to confer a competitive advantage to engrafted bone marrow (20, 21). We also observed an increase of GFP^+ cells in mice transplanted with hematopoietic progenitors ectopically expressing miR-125b, suggesting an effect on proliferation and an inhibition of apoptosis.

Some miR-125b targets have been identified and most of them are involved in apoptosis. Indeed, miR-125b targets the proapoptotic Bak1 and Bmf transcripts (14, 15). P53 is also a target of miR-125b in humans but the binding site in the 3′ UTR of p53 is not conserved in mouse (13). However, many other targets of miR-125b are in the p53 pathway and are conserved in mouse and man, as well as in other vertebrates (13). MicroRNA-125 overexpression blocks apoptosis in Ba/F3 cells and in primitive murine hematopoietic cells in vitro (19, 23). Of note, Klusmann et al. showed that miR-125b increases the proliferation and self-renewal of human and mouse megakaryocytic progenitors and megakaryocytic/erythroid progenitors without affecting their differentiation (19). In our transplanted mice, we didn't observe any difference in platelet number, but all of the mice transplanted with progenitors overexpressing miR-125b displayed macrocytic anemia. Given the fact that miR-125b overexpression was found in patients with myelodysplasia and the fact that the majority of patients with myelodysplasia display a macrocytic anemia, this mouse model could be a good model to study the mechanism involved in myelodysplastic syndromes.

We have also demonstrated that miR-125b overexpression accelerates the oncogenicity of the BCR-ABL fusion protein. It is well known that even strong oncogenes like BCR-ABL need a secondary event to transform a cell or to promote leukemia. Indeed, p53 has been shown to contribute to the blastic transformation of p210-expressing hematopoietic cells in vivo (24, 25). MicroRNA-125b profiling in tumor cells of patients with different malignancies would be useful to decipher the exact role of miR-125b as a second event in oncogenesis. A recent study showed that miR-125b overexpression is associated with the TEL-AML1 fusion protein in ALL patients and it confers a survival advantage in in vitro experiments (23). Furthermore, Zhang et al. showed that miR-125b is highly expressed in pediatric AML and in particular in patients with the translocation PML-RARA (26).

In summary, we report here that miR-125b is an oncomiR able both to induce primary lymphoid or myeloid leukemia in mice and to confer a proliferative advantage to BCR-ABL leukemic cells. As a driver mutation or a secondary event, miR-125b could be a promising therapeutic target in the treatment of leukemia.

Materials and Methods

Plasmid and Virus Production. To express miR-125b, we constructed the XZ-miR-125b-IRES-GFP plasmid by cloning a 408 bp genomic fragment containing the mouse precursor sequence of miR-125b-1, which was amplified by PCR using the forward primer 5′-ccgctcgagcccAGGTGTAGGGAGCCAGGATGTA-3′ and reverse primer 5′-ggaattcttaattaaaaaATGGAAGCCTCAAGGGTGTATT-3′, into the XZ vector, as previously described (27). The MSCV-p210-pac plasmid, allowing the expression of the BCR-ABL fusion gene, was a generous gift from Saghi Ghaffari, Mount Sinai Medical School, New York, NY. The 293T cells were transfected using Fugene with the retroviral constructs and the pCL-Eco plasmid at a ratio 1:1. Retroviral supernatant was harvested at 48, 72, and 96 h posttransfection.

Mice and Transplantation Assays. All animal experiments were performed with the approval of the Massachusetts Institute of Technology Committee on Animal Care. BCR-ABL bone marrow transplantations were performed as previously described with some modifications (28). Briefly, 5 d before bone marrow harvest, male BALB/c (The Jackson Laboratory) mice were injected with 150 mg/kg of 5-FU. Bone-marrow cells from 5-FU–treated BALB/c donor mice (6–8 wk old) were spin-infected for 2 h at a concentration of 1×10^6 cells/mL with the BCR-ABL retroviral supernatant plus 5 μg/mL of polybrene. Cells were cultured overnight in DMEM containing 5% of WEHI supernatant, 56 ng/mL of stem-cell factor, 12 ng/mL of IL-6, and 7 ng/mL of IL-3. The cells were resuspended for a first round of spin-infection with XZ or XZ-125b retroviral supernatant for 2 h. After an overnight culture and a second round of spin-infection with XZ or XZ-125b retroviral supernatant, cells were washed and resuspended in PBS containing 2% FBS. For each condition, female BALB/c recipient mice lethally irradiated (two doses of 450 rad, each dose administrated 4 h apart) were retro-orbitally injected with 4×10^5 cells. Our results are from 20 mice per group from three independent injections.

Lineage negative (Lin^-) fetal liver cells from 14.5 C57BL/6 mice (CD45.2) were enriched using the Mouse Hematopoietic Progenitor (Stem) Cell Enrichment Set (BD Biosciences) following the manufacturer's instructions. Briefly, fetal liver cells were stained with biotinylated monoclonal antibodies against CD3, CD11b, B220, Gr-1, and Ter-119. After 15 min of incubation, the cells were washed and incubated with streptavidin-conjugated with magnetic beads for 30 min on ice. The Lin^- cells were harvested by using AutoMacs magnetic separation and spin-infected during 2 h at $1,600 \times g$ 37 °C with XZ or XZ-125b retroviral supernatant plus 5 μg/mL of polybrene. Cells were then cultured overnight in IMDM, 20 ng/mL scf, 25 ng/mL FLT3-L, and 10 ng/mL IL-6 before a second round of spin-infection. Next, 1×10^6 cells were retro-orbitally injected into lethally irradiated (10 Gy) recipient mice (C57BL/6 CD45.1, NCI). Peripheral blood was harvested once a month for CBC and flow cytometry analysis. Mice were killed when moribund. Blood, bone marrow, and spleen were harvested and processed into single-cell suspensions. Samples of spleen, kidney, liver, lung, and bone were fixed in 10% neutral-buffered formalin for paraffin sectioning. Our results are from 20 mice per group from three independent injections.

RNA Extraction and RT-PCR. Total RNA was extracted according to the TRIzol method (Invitrogen). BCR-ABL transcripts were reverse-transcribed and amplified with the OneStep RT-PCR kit (Qiagen) using the forward 5′-AGCATGGCCTTCAGGGTGCACAGCCGCAACGGCAA-3′ and reverse 5′-TCACTGGGTCCAGCGAGAAGGTTTTCCTTGGAGTT-3′ primers.

Quantitative RT-PCR on MicroRNA. Reverse-transcription was performed using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. Quantitative PCR was performed on an ABI7900 (Applied Biosystems) using TaqMan MicroRNA Assays (Applied Biosystems). The presented data correspond to the mean of $2^{-\Delta\Delta Ct}$ from two independent reactions, normalized to the Sno202 reference transcript.

Flow Cytometry. Peripheral blood cells and single-cell suspensions from bone marrow and spleen were treated with ammonium chloride to lyse red cells (Stemcell Technologies). All cells were blocked with anti-mouse CD16/CD32 (FcγIII/II receptor; BD Pharmingen) and stained with APC anti-CD45.2 (eBiosciences) and the following lineage markers: Pacific Blue anti-B220 (eBiosciences) for B cells, PE anti-CD8 (BD Pharmingen) and PE-Cy7 anti-CD4 (eBiosciences) for T cells, and PE anti-CD11b (BD Pharmingen) and PE-Cy7 anti-Gr1 (eBiosciences) for myeloid cells. Data were analyzed on a BD LSR II flow cytometer by gating on CD45.2-positive cells.

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