miR-125b-2 is a potential oncomiR on human chromosome 21 in megakaryoblastic leukemia

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Children with trisomy 21/Down syndrome (DS) are at high risk to develop acute megakaryoblastic leukemia (DS-AMKL) and the related transient leukemia (DS-TL). The factors on human chromosome 21 (Hsa21) that confer this predisposing effect, especially in synergy with consistently mutated transcription factor GATA1 (GATA1s), remain poorly understood. Here, we investigated the role of Hsa21-encoded miR-125b-2, a microRNA (miRNA) overexpressed in DS-AMKL/TL, in hematopoiesis and leukemogenesis. We identified a function of miR-125b-2 in increasing proliferation and self-renewal of human and mouse megakaryocytic progenitors (MPs) and megakaryocytic/erythroid progenitors (MEPs). miR-125b-2 overexpression did not affect megakaryocytic and erythroid differentiation, but severely perturbed myeloid differentiation. The proproliferative effect of miR-125b-2 on MEPs accentuated the Gata1s mutation, whereas growth of DS-AMKL/TL cells was impaired upon miR-125b repression, suggesting synergism during leukemic transformation in GATA1s-mutated DS-AMKL/TL. Integrative transcriptome analysis of hematopoietic cells upon modulation of miR-125b expression levels uncovered a set of miR-125b target genes, including DICER1 and ST18 as direct targets. Gene Set Enrichment Analysis revealed that this target gene set is down-regulated in DS-AMKL patients highly expressing miR-125b. Thus, we propose miR-125b-2 as a positive regulator of megakaryopoiesis and an oncomiR involved in the pathogenesis of trisomy 21-associated megakaryoblastic leukemia.

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and apoptosis, by negatively regulating gene expression (for review, see Carthew and Sontheimer 2009). miRNAs are transcribed as primary miRNAs [pri-miRNAs] and processed in the nucleus by the RNase III endonuclease DROSHA to liberate ~70-nt stem loops, the precursor miRNA [pre-miRNA]. The pre-miRNAs are then exported from nucleus to cytoplasm by Exportin 5/RanGTP, where further cleavage by the second RNase III enzyme DICER releases the mature miRNAs. miRNAs bind to their target mRNAs by direct base-pairing, and either repress gene translation or induce mRNA cleavage or degradation (Carthew and Sontheimer 2009). In the hematopoietic system, several miRNAs, including miR-150, miR-223, and miR-181, regulate lineage decisions and cellular maturation (Chen et al. 2004; Xiao et al. 2007; Johnnids et al. 2008).

Deregulation of miRNA expression has been linked to the initiation, progression, and metastasis of human malignancies (He et al. 2005; Lu et al. 2005). Human and mouse cancers are characterized by a global reduction of mature miRNA levels compared with normal tissues (Lu et al. 2005; Kumar et al. 2007). miR-125b-2 resides along with four other known miRNAs [miR-99a, let-7c, miR-155, miR-802] on Hsa21 (Fig. 1A; Landgraf et al. 2007). Recent reports suggested that miR-125b might act as an oncogene as well as a tumor suppressor, depending on the cellular context. In prostate cancer cells, high expression levels of miR-125b stimulate androgen-independent growth that is mediated partially by down-regulation of Bak1 (Shi et al. 2007), whereas in breast cancer, high expression levels of miR-125b mediate down-regulation of ERBB2 [HER2] and ERBB3 [HER3], thereby suppressing tumor growth (Scott et al. 2007). The homolog miR-125b-1 is involved in translocatations found in precursor B-cell acute lymphoblastic leukemia [pre-B ALL] and myelodysplastic syndrome [MDS] (Sonoki et al. 2005; Bousquet et al. 2008). However, the role of Hsa21-encoded miR-125b-2 in leukemogenesis has not been defined.

In this study, we investigated the role of Hsa21-encoded miR-125b-2 in hematopoiesis and leukemogenesis. Using a genetic approach, we demonstrated that, in both murine and human contexts, overexpression of miR-125b-2 led to specific hyperproliferation and enhanced self-renewal capacity of megakaryocytic progenitors [MPs] and megakaryocytic/erythroid progenitors [MEPs], without affecting their normal differentiation. This effect was aggravated further in cooperation with the oncogenic Gata1s mutation. Integrative transcriptome analysis, together with experimental validation, revealed target genes of miR-125b in the hematopoietic system, including DICER1 and ST18 as direct targets. We showed that miR-125b was highly expressed in DS-AMKL blasts, whereas the identified target genes of miR-125b were down-regulated. Thus, our study supports a role of miR-125b-2 in the regulation of megakaryopoiesis and in the pathogenesis of trisomy 21-associated megakaryoblastic leukemia, in cooperation with GATA1s. We provide evidence that miR-125b-2 exerts its oncogenic potential by blocking post-transcriptional miRNA processing through repression of DICER1 expression and by inhibiting tumor suppressors, such as ST18.

Results

miR-125b is up-regulated in DS-AMKL and DS-TL

To interrogate a potential role for Hsa21-encoded miR-125b-2 in trisomy 21-associated megakaryoblastic leukemia [Fig. 1A], we first measured expression levels of miR-125b in sorted leukemic blasts from patients with DS-AMKL [n = 5], DS-TL [n = 4], non-DS-AMKL [n = 3], and AML FAB M5 [AML M5; n = 2], and in CD34+HSPCs [hematopoietic stem and progenitor cells] [n = 2] and megakaryocytes [BM-Meg; n = 1] from healthy donors. Data are presented as means ± standard error of the mean (SEM) normalized to CD34+HSPCs. *P < 0.05.

Figure 1. Hsa21-encoded miR-125b is up-regulated in AMKL patient samples. (A) Schematic diagram showing the location of miR-125b-2 and four other miRNAs [miR-99a, let-7c, miR-155, and miR-802] on human Hsa21 [http://www.ensembl.org]. (B) The expression level of miR-125b was analyzed by qRT-PCR in sorted leukemic blasts from patients with DS-AMKL (n = 5), DS-TL (n = 4), non-DS-AMKL (n = 3), and AML FAB M5 (AML M5; n = 2), and in CD34+HSPCs (n = 2) and megakaryocytes [BM-Meg; n = 1] from healthy donors. Data are presented as means ± standard error of the mean (SEM) normalized to CD34+HSPCs. *P < 0.05.
Klusmann et al.

differentiation, as the expression level of miR-125b in megakaryocytic cells was similar to that in CD34+ HSPCs. Expression of miR-125b was threefold higher in DS-AMKL than non-DS-AMKL.

Overexpression of miR-125b-2 increases proliferation and self-renewal of MPs

Overexpression of miR-125b in AMKL, and specifically in DS-AMKL and DS-TL, suggests a potential role of Hsa21-encoded miR-125b-2 in AMKL pathogenesis. To test the consequences of miR-125b-2 overexpression on megakaryocyte development, we transduced mouse MPs from fetal livers (FLs) of embryonic day 12.5 (E12.5) embryos with retroviruses [MSCV-based] containing the genomic region of miR-125b-2 or control empty vectors [LPIG containing only the miR-30 backbone [LMPIC] [Dickins et al. 2005], or MSCV-Puro]. We chose to transduce fetal progenitors because we showed previously that progenitor cells at this stage of development are the likely cells of origin for transformation in DS-AMKL [Li et al. 2005]. The level of mature miR-125b attained in transduced cells was comparable with that seen in the DS-AMKL cell line (CMK), as confirmed by Northern blot (Supplemental Fig. S1A). Following transduction, we performed megakaryocytic colony-forming assays. Retroviral overexpression of miR-125b-2 markedly accelerated the proliferation of MPs, as demonstrated by increased sizes and numbers of the megakaryocyte colony-forming units (CFU-MKs) [Fig. 2A–C; Supplemental Fig. S1B]. Megakaryocytic differentiation proceeded normally with EPO (erythropoietin), SCF (stem cell factor), IL-3 (interleukin-3), and IL-6 (interleukin-6), which support erythroid burst-forming units (BFU-E) and other CFUs of FL cells. In the third replating, whereas miR-125b-2-transduced FL progenitors mainly formed CFU-MKs (66.4 ± 2.8%; or better described as CFU-MK/Es, since many of these CFU-MK colonies also contained hemoglobinized erythroid cells) [Fig. 2H,I], a fraction of all colonies were BFU-Es (17.5 ± 4.2%); or described as BFU-Es [Fig. 2H,I]. In contrast, the empty vector-transduced control cells failed to form CFU-MKs or BFU-Es. They exclusively formed CFUs of other lineages [BFU-other], mainly CFUs containing mast cells (CFU-mast) [Fig. 2H,I]. Concomitant with this observation, by fluorescence-activated cell sorting [FACS] analysis, we found ~39% of all cells from the third replating plate were immunophenotypically CD41+CD71+ [compared with ~0.3% from the control plate], and ~30% were CD41+CD71+ [erythroid precursors; control: ~0.3%] [Fig. 2J].

The observed BFU-Es might have originated from unipotent erythroid progenitors and not from MEPs. However, we did not observe individual BFU-Es in the first and second rounds of replating. Instead, we observed Thus, miR-125b-2 not only increases the proliferation of MPs, it also enhances their ability to self-renew.

As these effects might be species-specific, we tested whether overexpression exerts similar effects in human hematopoietic cells. We retrovirally overexpressed miR-125b-2 in CD34+ HSPCs. Similar to findings in murine cells, forced expression of miR-125b-2 markedly increased the formation of larger CFU-MKs without blocking megakaryocytic differentiation [Fig. 2E,F]. To further assess consequences of miR-125b-2 overexpression on megakaryocytic development, we transduced K562 cells with miR-125b-2. K562 cells, which do not express miR-125b [Supplemental Fig. S2A], are multipotent and differentiate along the megakaryocytic lineage upon induction with chemical compounds or genetic alteration [Tetteroo et al. 1984]. Forced expression of miR-125b-2 (~7.8-fold higher levels relative to endogenous miR-125b expression of CMK cells) [Supplemental Fig. S2A] led to increased CD61 and reduced glycoporphin A [GlyA] expression, indicating the initiation of megakaryocytic differentiation [Fig. 2G]. The mRNA expression of megakaryocytic markers was increased, whereas the expression of erythroid markers was decreased [Supplemental Fig. S2B]. The proliferation rate of miR-125b-2-transduced K562 cells was not markedly altered [data not shown].

Overexpression of miR-125b-2 increases proliferation and self-renewal of MEPs

In comparison with non-DS-AMKL, DS-AMKL and DS-TL represent a unique entity in that their blast cells frequently exhibit features of cells in the erythroid lineage. Since megakaryocytic and erythroid cells arise from a common precursor [MEP], DS-AMKL/TL may be originated from MEPs. To analyze a possible effect of miR-125b-2 on MEPs and the erythroid lineage, we performed serial replating assays of wild-type FL progenitors in the presence of TPO along with EPO (erythropoietin), SCF (stem cell factor), IL-3 (interleukin-3), and IL-6 (interleukin-6), which support erythroid burst-forming units (BFU-E) and other CFUs of FL cells. In the third replating, whereas miR-125b-2-transduced FL progenitors mainly formed CFU-MKs (66.4 ± 2.8%; or better described as CFU-MK/Es, since many of these CFU-MK colonies also contained hemoglobinized erythroid cells) [Fig. 2H,I], a fraction of all colonies were BFU-Es (17.5 ± 4.2%); or better described as BFU-Es [Fig. 2H,I]. In contrast, the empty vector-transduced control cells failed to form CFU-MKs or BFU-Es. They exclusively formed CFUs of other lineages [BFU-other], mainly CFUs containing mast cells [CFU-mast] [Fig. 2H,I]. Concomitant with this observation, by fluorescence-activated cell sorting [FACS] analysis, we found ~39% of all cells from the third replating plate were immunophenotypically CD41+CD71+ [compared with ~0.3% from the control plate], and ~30% were CD41+CD71+ [erythroid precursors; control: ~0.3%] [Fig. 2J].

The observed BFU-Es might have originated from unipotent erythroid progenitors and not from MEPs. However, we did not observe individual BFU-Es in the first and second rounds of replating. Instead, we observed
many large CFU-MKs with hemoglobinized erythroid cells (data not shown). Thus, these data argue that miR-125b-2 can expand early hematopoietic progenitor cells (i.e., MEPs) with the potential to differentiate along the megakaryocytic and erythroid lineages without affecting their differentiation.

Overexpression of miR-125b-2 cooperates with Gata1s mutation

GATA1s mutations are consistently present in leukemic blasts of children with DS-AMKL and DS-TL. In the knock-in mouse model, Gata1s increases the proliferation of FL MPs in vitro and in vivo (Li et al. 2005). To test a potential synergistic function of Gata1s and miR-125b-2, we transduced Gata1s mutant FL cells with miR-125b-2 or the empty vector control and performed serial replating assays. Consistent with previous results (Li et al. 2005), we observed an increase in the number and size of CFU-MKs from Gata1s mutant FL progenitors and an enhanced replating efficiency in comparison with their wild-type counterparts [Fig. 3A; Supplemental Fig. S3]. Strikingly, miR-125b-2-transduced Gata1s FL progenitors frequently

Figure 2. miR-125b-2 overexpression induced proliferation and differentiation of MPs and MEPs. (A) Microscopic [left, middle] and macroscopic [right] images of AChE-stained and unstained (Phase) CFU-MKs from miR-125b-2-transduced and empty vector-transduced [LMPIG or MSCV-puro] murine FL cells. The inset of the bottom middle panel shows proplatelet formation. Pictures here are representative images from n = 3 independent experiments. (B,C) Megakaryocytic colony-forming assay of miR-125b-2-transduced, mutated miR-125b-2-transduced [mut1], and empty vector-transduced [LMPIG; control] wild-type FL MPs using methocellulose-based assays in the presence of 20 ng/mL TPO. (B) Diagram and statistics showing the number of CFU-MKs per 10^4 plated cells. Data of replicates from one of two independent experiments are shown as means ± SD. (*) P < 0.05. (C) Diagram and statistics showing the number of cells per CFU-MK. Data from replicates from one representative experiment are shown as means ± SD. (*) P < 0.05. (D) Diagram and statistics showing the number of CFU-MKs per 10^4 plated cells in serial replating of miR-125b-2-transduced and empty vector-transduced [LMPIG; control] wild-type FL progenitors using methocellulose-based assays. Data from replicates from one of two independent experiments are shown as means ± SD. (*) P < 0.05. (E) Diagram and statistics showing the number of CFU-MKs per 10^4 miR-125b-2-transduced and empty vector-transduced [MIGR1; control] human CD34^+ HSPCs. Data from n = 2 independent experiments are shown as means ± SD. (*) P < 0.05. (F) Representative images of immunohistochemically stained (CD41) and hematoxylin and eosin-stained (HE) colony-forming assays as shown in E. (G) CD61 and GlyA expression in miR-125b-2-transduced and empty vector-transduced K562 cells. The representative FACS profiles from one of two independent experiments are shown. (Blue line) Empty vector (LMPIG; control); (red line) miR-125b-2. (H) Representative microscopic images showing unstained (Phase) CFUs (CFU-Mast, BFU-E, and CFU-MK/E) from miR-125b-2-transduced and empty vector-transduced [MSCV-puro; control] murine wild-type FL cells from the third replating using methocellulose-based assays in the presence of TPO, SCF, EPO, IL-3, and IL-6. (I,J) Diagrams showing the relative distribution of CFUs [I] and the percentages of CD71^+CD41^− and CD71^+CD41^+ cells [J] as assessed by flow cytometry in the third replating of miR-125b-2-transduced and empty vector-transduced [MSCV-puro] murine wild-type FL cells as shown in H.
formed larger CFU-MKs than *Gata1*-only CFU-MKs throughout the serial replating experiment (Supplemental Fig. S3). In addition, the number of CFU-MKs from *miR-125b-2*-transduced *Gata1* FL progenitors increased constantly in each replating and was significantly higher in the third replating in comparison with the empty vector-transduced *Gata1* cells (Fig. 3A,B), suggesting a synergy of *Gata1* and *miR-125b-2* to further enhance proliferation and self-renewal of FL MPs. Consistent with this, we also observed increased proportions of CD41+ and CD71+ cells in replating plates with *miR-125b-2*-transduced *Gata1* FL progenitors in comparison with empty vector-transduced control cells (Fig. 3C). In addition, we did not observe BFU-E colonies emerging from replating assays from *miR-125b-2*-transduced *Gata1* FL progenitors in comparison with *miR-125b-2*-transduced wild-type FL progenitors (data not shown). This is expected, since *Gata1* mutation impairs fetal erythropoiesis, as we demonstrated previously in *Gata1* knock-in mice (Li et al. 2005).

**Down-regulation of miR-125b in DS megakaryocytic leukemia cells inhibits their proliferation**

To test dependency of proliferation of megakaryoblastic leukemia cells on continuous expression of *miR-125b*, we transfected CMK [DS-AMKL] and M-07 [non-DS-AMKL] cell lines with chemically modified oligonucleotides complementary to the sequence of *miR-125b* [anti-*miR-125b*]. *miR-125b* is highly expressed in both cell lines, as confirmed by Northern blot and quantitative RT–PCR (qRT–PCR), whereas expression of *miR-125b* is nearly undetectable in the presence of anti-*miR-125b* (Supplemental Figs. S2A, S4A). The number of viable cells was reduced upon transfection of anti-*miR-125b* in comparison with cells transfected with a nonsilencing control oligonucleotide (Fig. 3D). In contrast, K562 cells, which do not express *miR-125b*, did not show a similar response (Fig. 3D, Supplemental Fig. S2A). The effect was caused mainly by reduced proliferation, as indicated by cell cycle analysis with less BrdU* cells in S phase (Fig. 3E; Supplemental Fig. S4B,C). To investigate this effect in primary human cells, we transduced DS-TL leukemic blasts and CD34⁺-HSPCs with anti-*miR-125b*. Similar to the cell lines, DS-TL leukemic blasts ceased to proliferate (Fig. 3D), in contrast, the number of CD34⁺-HSPCs, which express only low levels of *miR-125b* in comparison with DS-TL leukemic blasts (Fig. 1B), was not altered significantly (Fig. 3D).

In conclusion, proliferation of DS-AMKL cell lines and primary DS-TL leukemic blasts is dependent on expression of *miR-125b*, and the hyperproliferative phenotype can be reverted upon down-regulation of *miR-125b*.

**miR-125b-2 perturbs myeloid differentiation of HSPCs**

*miR-125b-2* overexpression increases the proliferation of MEPs and is required for growth of DS-AMKL cell lines, as well as primary DS-TL blasts. To investigate a broader role of *miR-125b-2* in hematopoiesis, we transduced HSPC-enriched mononuclear cells from the bone marrow [BM] of adult mice. The number of CFUs of the *miR-125b-2*-transduced BM cells exceeded the number of the control CFUs [Fig. 4A]. Three major types of CFUs were present in the *miR-125b-2*-transduced BM cells: two types of large CFUs [type 1A and 1B], and one type of medium-sized, compact clusters [type 2] [Fig. 4B], as described previously for MLL-AF9-transduced myeloid cells [Johnson et al. 2003]. We picked individual CFUs from each type and analyzed their cell composition by cytospin and flow cytometry [FACS]. Type 1A CFUs contained mainly Mac-1-high macrophages and type 1B...
CFUs contained mast cells [CFU-other] (Fig. 4A,B). The vast majority of the CFUs (type 2) displayed a unique colony morphology and contained a mixture of granulocytes, macrophages, and immature myeloblasts (CFU-L) (Fig. 4A,B). Cells in these colonies were largely Mac-1low/mid, whereas 10% of cells also expressed Gr-1. The cells stained negative for other markers, such as Ter-119, Sca-1, c-kit, CD3, and B220 (data not shown).

In FL MPs/MEPs, enforced miR-125b-2 expression led to increased self-renewal with enhanced replating efficiency. To investigate the self-renewal capacity in miR-125b-2-transduced BM HSPCs, we performed serial replating assays. In contrast to MPs/MEPs, the number of CFUs from miR-125b-2-transduced BM HSPCs decreased significantly after the first replating (Fig. 4C). These data indicate that overexpression of miR-125b-2 specifically increases the self-renewal capacity of FL MPs and MEPs, but not the adult BM HSPCs.

To test if high levels of miR-125b-2 perturb human myelopoiesis, we retrovirally overexpressed miR-125b-2 in CD34+ HSPCs. In colony-forming assays, we observed a marked increase in the number and size of CFUs upon miR-125b-2 transduction in comparison with empty vector- and mutated miR-125b-2-transduced control cells.
Colonies exhibited unique, abnormal morphology, and contained a mixture of immature cells (CFU-L) (Fig. 4E).

During granulocytic differentiation, immature myeloblasts differentiate through the stages of promyelocytes, myelocytes, and metamyelocytes to become granulocytes in the presence of granulocyte colony-stimulating factor (G-CSF). As expected, when we cultured empty vector-transduced CD34^{+}-HSCPs in granulocytic differentiation media, we observed a gradual reduction of immature promyelocytes and an increase of more mature granulocytic cells (myelocytes, metamyelocytes, and granulocytes) with time in culture. In contrast, forced expression of miR-125b-2 led to a 2.2-fold increase of the absolute number of cells in the culture (Fig. 4H), accompanied by an increase of the proportion of promyelocytes, whereas mature neutrophils and macrophages were greatly underrepresented, as assessed by differential white blood cell [WBC] counts (Fig. 4J]. Only basophils were seen in the culture (Fig. 4I). Consistent with these data, we observed an increase of CD33^{+} promyelocytes upon forced miR-125b-2 expression, whereas the mature granulocytes in the control were CD33^{+} (Supplemental Fig. S5A]. Consistent with those data obtained in murine BM-HSCPs, the replating efficiency was not increased in human CD34^{+}-HSCPs (Supplemental Fig. S5B]. Neither the myeloid differentiation block nor the increased proliferation of CD34^{+}-HSCPs could be observed upon forced expression of miR-125b-2, mutated in the seed region, validating the specificity of the observed effects of miR-125b-2 (Supplemental Fig. S5C–F).

These data indicate that miR-125b-2 overexpression in HSCPs enhances their proliferation and blocks myeloid differentiation at the promyelocyte stage without enhancing replating efficiency.

Identification of miR-125b target genes in hematopoietic cells by integrative bioinformatic analysis

To identify target mRNAs of miR-125b in the hematopoietic system, we performed integrative bioinformatic analysis. We performed global microarray gene expression profiling of miR-125b-2-overexpressing HSCPs [miR-125b-2 vs. empty vector], as well as miR-125b knockdown AMKL cell lines [anti-miR-125b vs. control]. We reasoned that target genes of miR-125b would carry a miR-125b-binding site in the 3′UTR [untranslated region] [predicted by TargetScan] [Lewis et al. 2005; Grimson et al. 2007], and would be inversely regulated after overexpression or repression of miR-125b. Using dChip software [Li and Wong 2001], we identified 56 probes, representing 53 genes, among the putative miR-125b target gene list [as predicted by TargetScan]. These genes were up-regulated in the AMKL cell lines upon miR-125b knockdown and down-regulated in HSCP upon miR-125b-2 overexpression [Fig. 5A; Supplemental Fig. S6]. The most represented Gene Ontology categories of the 53 target genes, as indicated by the DAVID bioinformatics database [Dennis et al. 2003], include RNA binding, cell communication, intracellular signaling cascade, and regulation of biological processes [Supplemental Table S2]. The microarray data were confirmed for the candidate target genes DICER1 and ST18 [suppression of tumorigenicity 18] by qRT–PCR [Supplemental Fig. S7A]. The knockdown of DICER1 upon overexpression...
of miR-125b-2 in K562 cells was validated further by Western blot (Supplemental Fig. S7B).

We next sought to verify a direct regulation of these two target genes by miR-125b-2 using a luciferase reporter assay. ST18 has one predicted target site in the 3’UTR, and DICER1 has one site in the 3’UTR and one conserved site (17 genomes) within the ORF, as described previously [Forman et al. 2008]. We subcloned the 3’UTRs and the ORF containing the miR-125b-binding site of these transcripts into the luciferase-based reporter vector [pMIR-REPORT] and cotransfected the reporter constructs in the HEK293T cells with pMIGR1-mut (mutated miR-125b-2) or pMIGR1-miR-125b-2. miR-125b-2 expression repressed the relative luciferase activity of reporter constructs containing ST18 3’UTR, DICER1 3’UTR, and DICER1 ORF [Fig. 5B] in comparison with the mutated miR-125b-2. This shows that both ST18 and DICER1 are direct targets of miR-125b-2.

RNAi-mediated knockdown of miR-125b target genes DICER1 and ST18 recapitulates the hyperproliferative effect of miR-125b-2

We investigated further whether the phenotypes elicited upon overexpression or reduction of miR-125b levels might be reproduced by modulating expression of its target genes. The association of reduced DICER1 and ST18 expression levels and cancer has been described previously [Jandrig et al. 2004; Karube et al. 2005; Kumar et al. 2009]. We transduced human CD34+ HSPCs with a retrovirus (LMPIG) expressing shRNAs directed to DICER1 [shDICER] or ST18 [shST18], and confirmed knockdown by qRT–PCR [data not shown]. Repression of DICER1 or ST18 increases the number of CFUs by 1.4-fold [Fig. 5C]. The results were validated using two (ST18) and three (DICER1) different shRNAs, excluding the possibility of off-target effects (Supplemental Fig. S7C). In accordance with these data, when we repressed Dicer1 expression in mouse FL cells with a MSCV-based retrovirus expressing shRNA directed to Dicer1, we observed hyperproliferative, AChE-positive CFU-MKs in the colony-forming assay [Fig. 5D, Supplemental Fig. S7D].

Thus, we show by integrative analysis of the transcriptome of hematopoietic cells that miR-125b-2 may exert its proliferative effect on hematopoietic progenitor cells by repression of DICER1 expression and by inhibiting the tumor suppressor ST18.

miR-125b target genes are repressed in DS-AMKL and DS-TL

To determine if the above-identified target genes of miR-125b are down-regulated in primary leukemic blasts highly expressing miR-125b, we analyzed the global gene expression profiles of patient samples for which both miR-125b expression data and microarray gene expression data were available. Pearson’s correlation within the Gene Set Enrichment Analysis (GSEA) [Subramanian et al. 2005] was used to determine the degree of linear relationship between miR-125b expression and the expression of the identified miR-125b target gene set. We confirmed the negative relationship, and therefore down-regulation of the curated target genes set of miR-125b in samples highly expressing miR-125b [Fig. 6A]. To validate our findings, we performed GSEA using an independent, previously published data set that includes 23 patients with the DS-AMKL and 37 patients with non-DS-AMKL [Bourquin et al. 2006]. Similarly, we observed negative enrichment of the curated miR-125b target gene set in patients with DS-AMKL in comparison with those with non-DS-AMKL [Fig. 6B]. Of note was the fact that the genes DICER1 and ST18 were among the leading edge subsets [Fig. 6A,B].

Thus, our data show that miR-125b is highly expressed in DS-AMKL, whereas its putative target genes are down-regulated in DS-AMKL samples. These findings support a potential role of miR-125b-2 in the pathogenesis of DS-AMKL.

Discussion

We demonstrated that an Hsa21-encoded miRNA, miR-125b-2, plays a pivotal role in megakaryopoiesis by increasing proliferation and self-renewal of MPs and MEPs without affecting their differentiation. Our functional genetic studies further indicate that miR-125b-2 overexpression perturbs myeloid differentiation. In authentic human DS-AMKL, miR-125b-2 is highly overexpressed. In line with the observed impairment of proliferation of the DS-AMKL cell line and primary DS-TL blasts upon knockdown of miR-125b, and the demonstration of synergism between miR-125b-2 overexpression and Gata1s mutation in FL progenitors, our data indicate that miR-125b-2 is a potential oncomIR involved in the pathogenesis of trisomy 21-associated megakaryoblastic leukemia. Using an integrative bioinformatic approach, we analyzed the transcriptome after experimental alteration of miR-125b expression levels, and defined a set of target genes of miR-125b in hematopoietic cells that were down-regulated in DS-AMKL patient samples. The multidisciplinary approach we took underscores the validity of our findings and their biological relevance.

Interestingly, human Hsa21 carries several genes—including RUNX1, ERG, ETS2, and GABPA—that encode transcription factors serving as key regulators of megakaryocyte development [Elagib et al. 2003; Rainis et al. 2005; Pang et al. 2006]. It was shown recently that overexpression of ERG and ETS2 promoted megakaryopoiesis and synergized with GATA1 mutations [Gata1s or Gata1 knockdown] to immortalize fetal progenitors in vitro [Salek-Ardakani et al. 2009; Stankiewicz and Crispino 2009]. However, none of these genes is overexpressed in DS-AMKL and DS-TL samples in comparison with non-DS-AMKL samples [Bourquin et al. 2006]. Thus, it is uncertain whether the in vitro synergy between ERG or ETS2 overexpression and GATA1s mutation is directly relevant to the pathogenesis of DS-TL and DS-AMKL in vivo. Here we show that miR-125b-2 is another “megakaryocyte gene” on Hsa21 encoding for a different class of developmental regulators: miRNAs. In contrast to ERG and ETS2, miR-125b is indeed highly overexpressed in DS-AMKL and DS-TL blasts, whereas its expression is
low in CD34+ HSPCs, normal megakaryocytes, and AML FAB M5. Its overexpression appears closely associated with AMKL (especially with DS-AMKL/TL), and not with AML in general or with megakaryocytic differentiation. In fact, by miRNA profiling, it was shown that miR-125b was expressed in human MEPs, but was down-regulated during megakaryocyte differentiation [Lu et al. 2008]. Consistent with this, we observed dramatic expansion of MEPs in response to miR-125b-2 overexpression, indicating a potential role of this miRNA in controlling proliferation of MEPs. More importantly, we also observed synergy between overexpression of miR-125b-2 and Gata1s mutation to further increase proliferation and self-renewal of fetal MPs and MEPs (presumed cells of origin for DS-AMKL/TL), indicating potentially direct involvement of this miRNA in the pathogenesis of DS-AMKL/TL. Currently, factors leading to overexpression of miR-125b, particularly in DS-AMKL/TL blasts, remain to be defined. It will also be of great interest to determine whether miR-125b is overexpressed in human DS FL cells in the absence of GATA1s mutation, as FLs of healthy fetuses with DS have already contained hematopoietic progenitors preferentially differentiating toward megakaryocytic and erythroid lineages (Chou et al. 2008; Tunstall-Pedoe et al. 2008).

In mouse mesenchymal stem cells, exogenous miR-125b expression reduced proliferation and mediated differentiation [Mizuno et al. 2008], indicating a cell context-dependent role of miR-125b, inducing a proliferation program in one cell type and repressing a proproliferative program in another. The cell context-dependent difference of the effect of miR-125b is underscored by previous studies in prostate cancer cell lines. Despite the observation that down-regulation of miR-125b in AMKL cell lines and prostate cancer cell lines results in a proliferation arrest [Lee et al. 2005], the mechanism differs between cell types. The stimulation of androgen-independent growth of prostate cancer cells by miR-125b is at least partially mediated by down-regulation of BAK1, and thereby inhibition of apoptosis [Shi et al. 2007]. For AMKL cell lines, we failed to detect repression of BAK1 [data not shown]. Also, the recently defined target tumor suppressor p53 is not among our list of target genes [Le et al. 2009]. This argues for a differential gene expression program involved in crucial biological processes regulated by miR-125b.

Previous studies showed that both human and mouse cancers are characterized by a global reduction of mature miRNA levels compared with normal tissues [Lu et al. 2005; Kumar et al. 2007]. We identified and experimentally validated DICER1 as a direct target of miR-125b. Our results indicate a regulatory negative feedback loop between DICER1 and miR-125b-2. Production of mature miR-125b by DICER1 results in repression of DICER1 expression levels and, consequently, impaired overall miRNA processing (Fig. 7). Therefore, we propose a miRNA-mediated mechanism for low DICER1 expression levels, and provide an explanation for the observed failure to express mature miRNAs in many cancers. In concert with those data, we observed a global reduction of
miR-125b-2 in megakaryoblastic leukemia

miR-125b-2 in megakaryoblastic leukemia

miR-125b-2 in megakaryoblastic leukemia

miR-125b-2 in megakaryoblastic leukemia

Figure 7. Proposed model for the role of Hsa21-encoded miR-125b-2 in leukemogenesis. DICER1 and miR-125b-2 form a regulatory negative feedback loop. Production of mature miR-125b-2 by DICER1 results in repression of DICER1 expression levels and, subsequently, impaired overall miRNA processing. This miRNA-mediated mechanism for low DICER1 expression levels leads to a failure to express mature miRNAs essential for terminal hematopoietic differentiation. These oncogenic effects of miR-125b-2 can be enhanced further by the repression of tumor suppressors such as ST18.

miR-125b-2 in megakaryoblastic leukemia

miR-125b-2 in megakaryoblastic leukemia

miR-125b-2 in megakaryoblastic leukemia

miR-125b-2 in megakaryoblastic leukemia

Materials and methods

Patient samples

BM specimens from patients with non-DS-AMKL, DS-AMKL, DS-TL, or AML FAB M5, or from healthy donors were obtained from different children, and were provided by the collaborative AML-“Berlin–Frankfurt–Münster” Study Group (AML-BFM-SG, Münster, Germany). The study group performed a central review of the diagnosis, classification, and clinical follow-up of the patients. Mobilized CD34+ HSPCs from donors were positively selected by immunomagnetic labeling with corresponding magnetic cell-sorting beads (Miltenyi Biotech). All investigations were approved by the institutional review board and Ethics Committee of the Medical School Hannover, and informed consent was obtained according to local laws and regulations.

Mice

All studies involving mice were approved by the Children’s Hospital Boston Institutional Animal Care and Use Committee, and were performed in accordance with the relevant protocols. Gata1s mice were generated as described [Li et al. 2005].

Cell culture and transduction

All cell lines (CMK, M-07, and K562) were obtained from the German National Resource Center for Biological Material (DSMZ), and were cultured under the recommended conditions. Fetal MPs and BM cells from adult mice were processed, expanded, and infected as published previously [Li et al. 2005]. Culture, transduction on RetroNectin-coated (Takara) plates, and in vitro differentiation of CD34+ HSPC cells was performed as described [Klein et al. 2007; Skokowa et al. 2009]. The mouse stem cell virus (pMSCV)-based pMSCV-puro [Clontech], pMIGR1 [Pui et al. 1999], pMSCV-Puro-IRES-GFP (pLPIG), and pMSCV-Puro-IRES-GFP containing the miR-30 backbone (pLMPIG) vectors were used. Both pLPIG and pLMPIG retroviral vectors [Dickins et al. 2005] were generous gifts from Dr. Scott Lowe. pGIPZ vectors containing the shRNA sequence directed towards DICER1.
to DICER1 and ST18 were obtained from OpenBiosystems, and were cloned into the pLMPIC vector. Retroviral and lentiviral supernatant was prepared using standard protocols. Detailed procedures and vector design are provided in Supplemental Material.

Flow cytometry and cell sorting

Standard protocols were followed. Detailed procedures and antibodies are provided in Supplemental Material.

Colony-forming assays

Murine FL cells were plated in the MegaCult-C collagen-based system (StemCell Technologies) with TPO (5 ng mL\(^{-1}\)) and were stained for AChE activity in accordance with the manufacturer's instructions, or in Methocult M3231 methylcellulose medium (StemCell Technologies) in the presence of TPO (20 ng mL\(^{-1}\)), or in Methocult M3434 methylcellulose medium (StemCell Technologies) in the presence of TPO (20 ng mL\(^{-1}\)). BM cells from adult mice were assayed for their colony-forming activity in Methocult M3231 methylcellulose medium (StemCell Technologies) in the presence of erythropoietin (Epo; 3 U mL\(^{-1}\)), or in Methocult M3434 methylcellulose medium (StemCell Technologies) in the presence of Epo (3 U mL\(^{-1}\)). Colony-forming assays in accordance with the manufacturer's instructions (StemCell Technologies). For serial replating, cells from methocellulose-based colony-forming assays were harvested, washed in IMDM, counted, and seeded in the respective colony-forming assays at a density of 1 \(\times\) 10\(^4\) to 2 \(\times\) 10\(^5\) cells per plate.

Microarray and bioinformatics analysis

Microarray expression profiles were collected using Affymetrix Human Genome U133 Plus 2.0 chips, and were analyzed using dChip (Li and Wong 2001) and GSEA (Subramanian et al. 2005). For miRNA target gene prediction, we used the TargetScan 4.1 algorithm (Lewis et al. 2005; Grimson et al. 2007). Analysis of Gene Ontology categories was performed using DAVID bioinformatics database (Dennis et al. 2003). All microarray data have been deposited in NCBI's Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo) with GEO Series accession numbers GSE19680 and GSE19681.

miRNA expression studies

miRNA expression was measured on a 7900HT Fast Real-Time PCR System using a TaqMan Human MicroRNA Array version 1.0 (Applied Biosystems) in accordance with the manufacturer's instructions. For miRNA Northern blots, total RNA was separated in 15% polyacrylamide TBE/Urea gel, transferred to GeneScreen Plus transfer membrane (PerkinElmer), and UV-cross-linked in Stratalinker (Stratagene). Anti-miR-125b (Ambion) was labeled by \(\gamma\)-32P-ATP using the mirVana miRNA Detection Kit according to the manufacturer's instructions (Ambion) and was used as the probe. Standard procedures were followed for hybridization/washes and detection.

Transfection of 2'-O-methyl antisense oligonucleotides

2'-O-methyl antisense oligonucleotides labeled with Cy3 and directed against the mature miR-125b were chemically synthesized (BioSpring). Cells (1 \(\times\) 10\(^4\)) were electroporated (330 V, 10 msec) in 100 µL of medium containing 0.5 µg of 2'-O-methyl antisense oligonucleotides in a 4-mm electroporation cuvette using an EPI 2500 gene pulser (Fischer). Transfected cells were FACSc-sorted after 24 h as described above.

Western blot

Total cell lysis and Western blotting were performed using standard protocols as described previously (Li et al. 2007). Western blots were probed with the following antibodies: DICER1 (Abcam) and GAPDH (Abcam). Proteins were visualized with ECL (Amer sham) according to the manufacturer's protocols.

qRT–PCR

RNA for mRNA qRT–PCR was isolated using the RNeasy Mini Kit (QIAGEN). cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). For miRNA qRT–PCR, the QuantiTect SYBR Green PCR kit (QIAGEN) was used according to the manufacturer's specifications. For each 25-µL reaction mixture, 250 ng of cDNA were used. mRNA expression was normalized against expression of human GAPDH (QIAGEN) expression levels. RNA for miRNA qRT–PCR was isolated by use of miRana miRNA Isolation Kit (Ambion). cDNA Synthesis was performed with the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) according to the manufacturer's instructions. miRNA expression was quantified using TaqMan miRNA assays for hsa-miR-125b and RNU44d as the housekeeping gene (Applied Biosystems). Analysis was done on a StepOnePlus (Applied Biosystems) real-time thermal cycler.

Luciferase reporter assay

For reporter assays, the 3'UTRs or ORF fragment of the respective miRNAs containing the miR-125b-binding site were cloned from cDNA into pMIR-REPORT miRNA Expression Reporter Vector (Ambion). 293T cells (1 \(\times\) 10\(^3\)) were plated and cotransfected in 96-well plates by using FuGENE HD (Roche), according to the manufacturer's protocol, with 6.25 ng of the firefly luciferase report vector, 0.25 ng of the control vector containing renilla luciferase PGL4.7 (Promega), and 50 ng miRNA expression vector (pMiGR1-mir-125b-2 and pMiGR1-mut). Firefly and renilla luciferase activities were measured consecutively by using dual-luciferase assays (Promega) 48 h after the transfection. All experiments were performed in duplicates.

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References

Bourquin JP, Subramanian A, Langebrace C, Reinhardt D, Bernard O, Ballerini P, Baruchel A, Cave H, Dastugue N,
mirt-125b-2 in megakaryoblastic leukemia

Hasle H, et al. 2006. Identification of distinct cellular phenotypes in acute megakaryoblastic leukemia by gene expression profiling. Proc Natl Acad Sci 103: 3339–3344.

Bousquet M, Quelen C, Rosati R, Mansat-De Mas V, La Starza R, Bastard C, Lippet E, Talmant P, Lafage-Pochitaloff M, Leroux D, et al. 2008. Myeloid cell differentiation arrest by mirt-125b-1 in myelodysplastic syndrome and acute myeloid leukemia with the t(2;11)(p21;q23) translocation. J Exp Med 205: 2499–2506.

Carthew RW, Sontheimer EJ. 2009. Origins and mechanisms of miRNAs and siRNAs. Cell 136: 642–655.

Chen CZ, Li L, Lodish HF, Bartel DP. 2004. MicroRNAs modulate hematopoietic lineage differentiation. Science 303: 83–86.

Choi ST, Opalinska JB, Yao Y, Fernandes MA, Kalota A, Brooks JS, Choi JK, Gewirtz AM, Danet-Desnoyers GA, Nemiroof RL, et al. 2008. Trisomy 21 enhances human fetal erythro-megakaryocytic development. Blood 112: 4503–4506.

Dennis G Jr, Sherman BT, Hosack DA, Yang J, Gao W, Lane HC, Lampicki RA. 2003. DAVID: Database for annotation, visualization, and integrated discovery. Genome Biol 4: 3.

Dickins RA, Hemmat MT, Zilfou JT, Simpson DR, Ibarra I, Hannon GJ, Lowe SW. 2005. Probing tumor phenotypes using stable and regulated synthetic microRNA precursors. Nat Genet 37: 1289–1295.

Elagib KE, Racke FK, Mogass M, Khewattar R, Delechanty LL, Goldfarb AN. 2003. RUNX1 and GATA-1 cooperativity and cooperation in megakaryocytic differentiation. Blood 101: 4333–4341.

Forman JJ, Legesse-Miller A, Coller HA. 2008. A search for conserved sequences in coding regions reveals that the let-7 microRNA targets Dicer within its coding sequence. Proc Natl Acad Sci 105: 14879–14884.

Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP. 2007. MicroRNA targeting specificity in mammals: Determinants beyond seed pairing. Mol Cell 27: 91–105.

Hasle H. 2001. Pattern of malignant disorders in individuals with Down’s syndrome. Lancet Oncol 2: 429–436.

He L, Thomson JM, Hemmat MT, Hernando-Monco E, Mu D, Goodson S, Powers S, Cordon-Cardo C, Lowe SW, Hannon GJ, et al. 2005. A microRNA polycistron as a potential human oncogene. Nature 435: 828–833.

Holland LA, Lima CS, Cunha AF, Albuquerque DM, Vassallo J, Dickins RA, Hemmat MT, Zilfou JT, Simpson DR, Ibarra I, Hannon GJ, Lowe SW. 2005. Probing tumor phenotypes using stable and regulated synthetic microRNA precursors. Nat Genet 37: 1289–1295.

Jandrig B, Seitz S, Hinzmann B, Arnold W, Micheel B, Koeblke H, Siebert R, Schwartz A, Ruecker K, Schlag PM, et al. 2004. ST18 is a breast cancer tumor suppressor gene at human chromosome 8q11.2. Oncogene 23: 9295–9302.

Johnmids JB, Harris MH, Wheeler RT, Stehling-Sun S, Lam MH, Kirak O, Brummelkamp TR, Fleming MD, Camardo FD. 2008. Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. Nature 451: 1125–1129.

Johnson JJ, Chen W, Hudson W, Yao Q, Taylor M, Rabbitts TH, Kersey JH. 2003. Prenatal and postnatal myeloid cells demonstrate stepwise progression in the pathogenesis of MLL fusion gene leukemia. Blood 101: 3229–3235.

Karoie B, Tanaka H, Osada H, Tomida S, Tatematsu Y, Yanagisawa K, Yatabe Y, Takamizawa J, Mitsudomi T, et al. 2005. Reduced expression of Dicer associated with poor prognosis in lung cancer patients. Cancer Sci 96: 111–115.

Klein C, Grudzien M, Appaswamy G, Gernsheusen M, Sandrock I, Schaffer AA, Rathinam C, Boztug K, Schwinzer B, Rezaei N, et al. 2007. HAX1 deficiency causes autosomal recessive severe congenital neutropenia [Kostmann disease]. Nat Genet 39: 86–92.

Klusmann JH, Creutzig U, Zimmermann M, Dwozak M, Jorch N, Langebrake C, Pekrun A, Macakova-Reinhardt K, Reinhardt D. 2008. Treatment and prognostic impact of transient leukemia in neonates with Down syndrome. Blood 111: 2991–2998.

Kumar MS, Lu J, Mercer KL, Golub TR, Jacks T. 2007. Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nat Genet 39: 673–677.

Kumar MS, Pester RE, Chen CY, Lane K, Chin C, Lu J, Kirsch DG, Golub TR, Jacks T. 2009. Dicer1 functions as a haploinsufficient tumor suppressor. Genes & Dev 23: 2700–2704.

Landgraf P, Rusu M, Sheridan R, Sewer A, Iovino N, Aravin A, Pfeffer S, Rice A, Kamphorst AO, Landthaler M, et al. 2007. A mammalian microRNA expression atlas based on small RNA library sequencing. Cell 129: 1401–1414.

Lee MT, Teh C, Shyh-Chang N, Xie H, Zhou B, Korzh V, Lodish HF. Lim B. 2009. MicroRNA-125b is a novel negative regulator of p53. Genes & Dev 23: 862–876.

Lee YS, Kim HK, Chung S, Kim KS, Dutta A. 2005. Depletion of human micro-RNA miR-125b reveals that it is critical for the proliferation of differentiated cells but not for the down-regulation of putative targets during differentiation. J Biol Chem 280: 16635–16641.

Lewis BP, Burge CB, Bartel DP. 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120: 15–20.

Li C, Wong WH. 2005. Model-based analysis of oligonucleotide arrays: Expression index computation and outlier detection. Proc Natl Acad Sci 98: 31–36.

Li Z, Godinho FJ, Klussmann JH, Garriga-Canut M, Yu C, Orkin SH. 2005. Developmental stage-selective effect of somatically mutated leukemogenic transcription factor GATA1. Nat Genet 37: 613–619.

Li Z, Tognon CE, Godinho FJ, Yasaitis L, Hock H, Herschkowitz JJ, Lannon CL, Cho E, Kim SJ, Bronson RT, et al. 2007. ETV6-NTRK3 fusion oncogene initiates breast cancer from committed mammary progenitors via activation of API complex. Cancer Cell 12: 542–558.

Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, et al. 2005. MicroRNA expression profiles classify human cancers. Nature 435: 834–838.

Lu J, Guo S, Ebert BL, Zhang H, Peng X, Bosco J, Pretz J, Schlanger R, Wang YJ, Mak RH, et al. 2008. MicroRNA-mediated control of cell fate in megakaryocyte-erythrocyte progenitors. Dev Cell 14: 843–853.

Maligne S, Izraeli S, Crispino JD. 2009. Insights into the manifestations, outcomes, and mechanisms of leukemogenesis in Down syndrome. Blood 113: 2619–2628.

Mizuno Y, Yagi K, Tokuzawa Y, Kanaei-Katsuya Y, Suda T, Katagiri T, Fukuda T, Maruyama M, Okuda A, Amemiya T, et al. 2008. mir-125b inhibits osteoblastic differentiation by down-regulation of cell cycle. Biochem Biophys Res Commun 368: 267–272.

Mundschau G, Gurbuxani S, Gamis AS, Greene ME, Arceci RJ, Crispino JD. 2003. Mutagenesis of GATA1 is an initiating event in Down syndrome leukemogenesis. Blood 101: 4298–4300.

Pang L, Xue HH, Szalai G, Wang X, Wang Y, Watson DK, Leonard WJ, Blobel GA, Pomez M. 2006. Maturation stage-specific regulation of megakaryopoiesis by pointed-domain Ets proteins. Blood 108: 2198–2206.

Pui JC, Allman D, Xu L, DeRocco S, Karrn F, Bakkour S, Lee JY, Kadesch T, Hardy RR, Aster JC, et al. 1999. Notch1
expression in early lymphopoiesis influences B versus T lineage determination. *Immunity* **11**: 299–308.

Rainis L, Toki T, Pimanda JE, Rosenthal E, Machol K, Strehl S, Gottgens B, Ito E, Izraeli S. 2005. The proto-oncogene ERG in megakaryoblastic leukemias. *Cancer Res* **65**: 7596–7602.

Salek-Ardakani S, Smooha G, de Boer J, Schire NJ, Morrow M, Rainis L, Lee S, Williams O, Izraeli S, Brady HJ. 2009. ERG is a megakaryocytic oncogene. *Cancer Res* **69**: 4665–4673.

Scott GK, Goga A, Bhaumik D, Berger CE, Sullivan CS, Benz CC. 2007. Coordinate suppression of ERBB2 and ERBB3 by enforced expression of micro-RNA miR-125a or miR-125b. *J Biol Chem* **282**: 1479–1486.

Shi XB, Xue L, Yang J, Ma AH, Zhao J, Xu M, Tepper CG, Evans CP, Kung HJ, deVere White RW. 2007. An androgen-regulated miRNA suppresses Bak1 expression and induces androgen-independent growth of prostate cancer cells. *Proc Natl Acad Sci* **104**: 19983–19988.

Skokowa J, Lan D, Thakur BK, Wang F, Gupta K, Cario G, Brechlin AM, Schambach A, Hinrichsen L, Meyer G, et al. 2009. NAMPT is essential for the G-CSF-induced myeloid differentiation via a NAD⁺–sirtuin-1-dependent pathway. *Nat Med* **15**: 151–158.

Sonoki T, Iwanaga E, Mitsuya H, Asou N. 2005. Insertion of microRNA-125b-1, a human homologue of lin-4, into a rearranged immunoglobulin heavy chain gene locus in a patient with precursor B-cell acute lymphoblastic leukemia. *Leukemia* **19**: 2009–2010.

Stankiewicz MJ, Crispino JD. 2009. ETS2 and ERG promote megakaryopoiesis and synergize with alterations in GATA-1 to immortalize hematopoietic progenitor cells. *Blood* **113**: 3337–3347.

Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, et al. 2005. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci* **102**: 15545–15550.

Tetteroo PA, Massaro F, Mulder A, Schreuder-van Gelder R, dem Borne AE. 1984. Megakaryoblastic differentiation of proerythroblastic K562 cell-line cells. *Leuk Res* **8**: 197–206.

Tokumaru S, Suzuki M, Yamada H, Nagino M, Takahashi T. 2008. let-7 regulates Dicer expression and constitutes a negative feedback loop. *Carcinogenesis* **29**: 2073–2077.

Tunstall-Pedoe O, Roy A, Karadimitris A, de la Fuente J, Fisk NM, Bennett P, Norton A, Vyas P, Roberts I. 2008. Abnormalities in the myeloid progenitor compartment in Down syndrome fetal liver precede acquisition of GATA1 mutations. *Blood* **112**: 4507–4511.

Viswanathan SR, Daley GQ, Gregory RL. 2008. Selective blockade of microRNA processing by Lin28. *Science* **320**: 97–100.

Wechsler J, Greene M, McDevitt MA, Anastasi J, Karp JE, Le Beau MM, Crispino JD. 2002. Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. *Nat Genet* **32**: 148–152.

Xiao C, Calado DP, Galler G, Thai TH, Patterson HC, Wang J, Rajewsky N, Bender TP, Rajewsky K. 2007. MiR-150 controls B cell differentiation by targeting the transcription factor c-Myb. *Cell* **131**: 146–159.

Yang J, Siqueira MF, Behl Y, Alikhani M, Graves DT. 2008. The transcription factor ST18 regulates proapoptotic and proinflammatory gene expression in fibroblasts. *FASEB J* **22**: 3956–3967.
miR-125b-2 is a potential oncomiR on human chromosome 21 in megakaryoblastic leukemia

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