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The biology of human hematopoietic stem and progenitor cells in acute myeloid leukemia, aging and autologous transplantation

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CHAPTER 3

Downregulation of MEIS1 impairs long-term expansion of CD34+ NPM1-mutated acute myeloid leukemia cells

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Abstract

Acute myeloid leukemia (AML) with mutated NPM1 (NPMc^{+} AML) shows distinctive biological and clinical features and is a provisional entity in the 2008 WHO classification. It is currently unclear how mutant NPM1 contributes to leukemic transformation and which molecular mechanisms underlie self-renewal of stem cells in NPMc^{+} AML. In the present study it is demonstrated that mutated NPM1 is expressed in both the CD34^{+} as well as the CD34^{-} NPMc^{+} AML subfractions. Importantly, we observe that long-term expansion and self-renewal *in vitro* is retained within the CD34^{+}, but not the CD34^{-} subfraction. Large-scale gene expression profiling was performed on paired NPMc^{+} CD34^{+} and CD34^{-} fractions (n=10), on NPMwt CD34^{+} samples (n=33) and on normal bone marrow (NBM) CD34^{+} samples (n=24). These studies revealed a significantly increased expression of HOX genes and MEIS1 in NPMc^{+} CD34^{+} AML compared to NPMwt CD34^{+} AML and CD34^{+} NBM. Lentiviral shRNA-mediated downregulation of MEIS1 expression in primary CD34^{+} NPMc^{+} AML cells resulted in a significant inhibition of leukemic growth, demonstrating that MEIS1 is an important target gene in NPM1-mutated AML that is required for long-term proliferation of NPMc^{+} CD34^{+} AML cells.

Introduction

Mutations of the nucleophosmin gene (NPM1) occur in about 30% of all AML patients and cause aberrant accumulation of NPM1 in the cytoplasm of leukemic cells (NPMc$^+$ AML).[1] It is currently unclear how mutant NPM1 contributes to leukemic transformation and which molecular mechanisms underlie self-renewal of stem cells in NPMc$^+$ AML.

In most cases the leukemia initiating cells (LICs) have been found to reside in the CD34$^+$ fraction. Since the vast majority of the leukemic cells within NPMc$^+$ AML are negative for CD34, it remained unclear whether the generally accepted CD34$^+$ expression profile of LICs would also apply to NPMc$^+$ AML. Recently, this question has been addressed by two research groups. While Taussig et al. describe that the CD34$^-$ fraction of NPMc$^+$ AML contains cells that could engraft immunocompromised mice in all studied samples,[2] Martelli et al. showed that in most cases the CD34$^+$ but not CD34$^-$ cells generated NPMc$^+$ AML in immunocompromised mice.[3]

NPMc$^+$ AML has a unique gene expression profile, which is characterized by upregulation of HOX genes and MEIS1.[4;5] It has been hypothesized that the (re)activation of a stem cell-like HOX gene signature in NPMc$^+$ AML might contribute to the leukemic transformation, but formal proof for this concept is currently lacking.

Materials and methods

Patient material

AML blasts from peripheral blood (PB) or bone marrow (BM) were obtained from untreated patients diagnosed with AML after achieving informed consent. Mononuclear cells (MNC) were isolated by density gradient centrifugation using lymphoprep (PAA, Cölbe, Germany). CD34$^+$ and CD34$^-$ AML cells were selected by MoFLo sorting (Dako Cytomation, Carpinteria, CA, USA) using a CD34 PE-labeled antibody (BD Biosciences, San Jose, California, USA). Patients were considered to have mutant NPM1 if they had both a nuclear and cytoplasmic localization of NPM1 as demonstrated by immunohistochemistry on bone marrow biopsies. NPM1 mutations were also verified by sequencing the C-terminal region. As healthy control samples CD34$^+$ cells from healthy bone marrow (BM) material was used. The study protocol was approved by the Medical Ethical Committee of the University Medical Center Groningen.

Western blot analysis

Western blot analysis was performed using standard procedures. The rabbit polyclonal antibody (SIL-A) specifically recognizing the NPM1 mutant A protein (kindly provided by Prof. Falini, Institute of Hematology, University of Perugia, Perugia, Italy) was used in a concentration of 1:250 and

incubated overnight at 4ºC after blocking with 5% BSA in TBS-Tween (0.05%) for one hour. The mouse monoclonal antibody recognizing both wild type and mutant NPM1 (also a generous gift of Prof. Falini, University of Perugia) was used in a concentration of 1:300 and incubated for 2-3 hours at room temperature. The antibody against MEIS (clone 9.2.7) was obtained from Millipore (Amsterdam, The Netherlands). The β-actin antibody was obtained from Santa Cruz Biotechnology (Heidelberg, Germany). Secondary antibodies were obtained from DAKO (Glostrup, Denmark) and used in a concentration of 1:3000. The K562 cell line was used as a negative control and the OCI-AML3 cell line as a positive control for mutant NPM1.

Sequencing analysis

Total RNA was isolated using the RNeasy kit from Qiagen (Venlo, The Netherlands) according to the manufacturer's recommendations. For NPM1 mutation analysis RT-PCR was performed using the forward primer 5'-agcgccagtgaagaaatc-3' and the reverse primer 5'-cacggtagggaaagttctc-3'. PCR products were sequenced to identify the mutation.

Long-term AML cultures on MS5 stroma

Long-term AML cultures were performed as described previously (*van Gosliga et al. Exp Hematol 2007*). Briefly, for the long-term cultures 6 to 40 x 10^3 cells, sorted based on CD34 expression, were plated in a 12-wells plate precoated with MS5 stromal cells. Cells were expanded in LTC medium (α-minimum essential medium supplemented with heat-inactivated 12.5% fetal calf serum (Sigma, Zwijndrecht, The Netherlands), heat-inactivated 12.5% horse serum (Sigma), penicillin and streptomycin, 2 mM glutamine, 57.2 μM β-mercaptoethanol (Sigma) and 1 μM hydrocortisone (Sigma) supplemented with 20 ng/ml IL-3, 20 ng/ml granulocyte colony-stimulating factor (G-CSF) (Rhone-Poulenc Rorer, Amstelveen, The Netherlands) and 20 ng/ml thrombopoietin (TPO) (Kirin, Tokyo, Japan). Cultures were kept at 37 ºC and 5% CO2. Cultures were demi-depopulated weekly for analysis. After five weeks both suspension and trypsinized cobblestone area forming cells (CAFC) were harvested to initiate second co-cultures on a new stromal layer. Before replating CAFC were separated from old MS5 cells by MoFLo sorting based on human CD45 and/or CD34 expression. Both suspension and sorted CAFC cells were used in the replating experiments. Pictures of stromal co-cultures were taken using a Leica DM-IL microscope (Leica Microsystems, Rijswijk, The Netherlands) with a 40x/0.60 objective.

Lentiviral transductions

The lentivirus short hairpin RNA vector targeting both human as well as murine MEIS1 was obtained from Open Biosystems (Huntsville, AL; Accessionnumber: NM_002398; source ID: TRCN0000012526) and cloned into the pLKO.1 lentiviral vector containing green fluorescent protein (GFP). A control vector was made by cloning a scrambled (SCR) short hairpin into the pLKO.1 GFP

vector. Lentiviral particles were made by transient transfection of 293T cells with the lentiviral expression vectors and stable transduction of CD34^{+} AML cells was performed, both were extensively described previously (*Schepers et al. Blood 2007*).

Gene expression profiling

AML MNC fractions derived from PB or BM were sorted based on CD34 expression on a MoFlo. Total RNA was isolated using the RNeasy mini kit from Qiagen (Venlo, The Netherlands) according to the manufacturer's recommendations. RNA quality was examined using the Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). For real-time RT-PCR, cDNA was prepared and amplified using iQ SYBR Green supermix (Bio-Rad, Veenendaal, The Netherlands) in a MyIQ thermocycler (Bio-Rad), and quantified using MyIQ software (Bio-Rad) using RPL27 and HPRT as housekeeping genes. Genome-wide expression analysis was performed on Illumina (Illumina, Inc., San Diego, CA) BeadChip Arrays Sentrix Human-6 (46k probesets). Typically, 0.5-1 µg of mRNA was used in labeling reactions and hybridization with the arrays according to the manufacturer's instructions. Data were analyzed using the BeadStudio v3 Gene Expression Module (Illumina, Inc.) and Genespring (Agilent, Amstelveen, The Netherlands).

Statistical analysis

Clustering analyses were performed on the basis of gene expression profiles using Genespring GX10 software. For comparison of CD34^{+} NPMc^{+} versus NPMwt AML and NBM samples a random variance t-test was used. For this experiment differential expression was considered significant at $P<1E10^{-6}$. For comparison of CD34^{+} and CD34^{-} an unpaired two-sided t-test was used with equal variance and $P<0.001$.

Results and discussion

Characteristics of the AML samples used for the present study and an overview of the performed experiments are shown in Supplemental Table 1. In addition, a detailed materials and methods section is provided in the Supplementary Information. To investigate whether mutant NPM1 protein is present in both the CD34^{+} and CD34^{-} cell fractions of NPMc^{+} AML, cells of six AML samples were sorted based on CD34 expression (Figure 1A) and Western blot analysis was performed. Using an antibody that specifically recognizes NPM1 mutant protein and not the wild type protein,[6] it was demonstrated that mutant NPM1 protein was expressed in both the CD34^{+} and CD34^{-} AML cell fractions (Figure 1B), indicating that both the CD34^{+} and CD34^{-} subpopulation of NPMc^{+} AML belong to the leukemic clone. The lower expression level of NPM1 and NPM1 mutant in CD34^{-} cells might be a reflection of the more differentiated state of the CD34^{-} AML cells compared to CD34^{+} AML cells.[7]

The presence of the NPM1 mutation in the $CD34^+/CD38^-$ fraction of $NPMc^+$ AML was shown by Western blot analysis of two AML samples (Figure 1C).
To investigate whether long-term *ex vivo* expansion and self-renewal properties reside in the $CD34^+$ or $CD34^-$ population within $NPMc^+$ AML samples, cells were sorted based on CD34 expression and long-term co-cultures were initiated on MS5 bone marrow stromal cells.[8;9] No long-term expanding cultures could be established with $CD34^-$ $NPMc^+$ cells (Figure 1D). In contrast, when co-cultures were initiated with $CD34^+$ $NPMc^+$ cells a strong expansion (5-474 fold) was observed within the first five weeks in 80% of investigated cases (Figure 1D). This expansion was associated with the formation of cobblestone areas under the stromal layer within three weeks after plating (Figure 1E). The rate of expansion was independent of the presence of FLT3-ITD.
In order to assess self-renewal capacity of expanding cultures, serial replating experiments were performed. After five weeks of co-culture of AML cells on MS5, the leukemic suspension and adherent cells were isolated based on human CD45 expression and cells were replated onto new MS5 stroma. In 7 out of 9 investigated cases, which were initiated with $CD34^+$ $NPMc^+$ AML cells, replated cells were capable of initiating 2nd expanding cultures, whereby new leukemic cobblestone areas were formed underneath the stroma (Figure 1E). Indeed, some $CD34^+$ $NPMc^+$ AML cells could be maintained up to 20 weeks. The leukemic origin of the expanded replated cells was confirmed by demonstrating the presence of mutant NPM1 protein by Western blotting in three examined cases (data not shown). To investigate whether $CD34^+$ $NPMc^+$ cells can give rise to $CD34^-$ progeny, expanding $NPMc^+$ AML cases (n=5) were analyzed for the percentage of $CD34^+$ cells by FACS. Although the cultures were initiated with sorted $CD34^+$ cells with a purity of >95%, in all cases the percentage of CD34 dropped again to relatively low levels (ranging from 0.22-4%) within three weeks upon *in vitro* culturing, suggesting that a new hierarchy within the leukemic clone was formed. To further investigate this hierarchy, replating experiments were performed with cultured expanding cells again sorted based on CD34 expression before replating (n=4, Supplemental Table 1). Indeed, only $CD34^+$ cells were able to expand in a secondary and tertiary replating experiment and gave rise to $CD34^-$ progeny (Figure 1F). In contrast, $CD34^-$ cells were not able to expand after replating, even when 100,000 cells were replated. Annexin V/PI apoptosis assays showed that a number of $CD34^-$ replated cells went into apoptosis within four weeks after plating (6.7-36% Annexin V-positive cells) (data not shown).

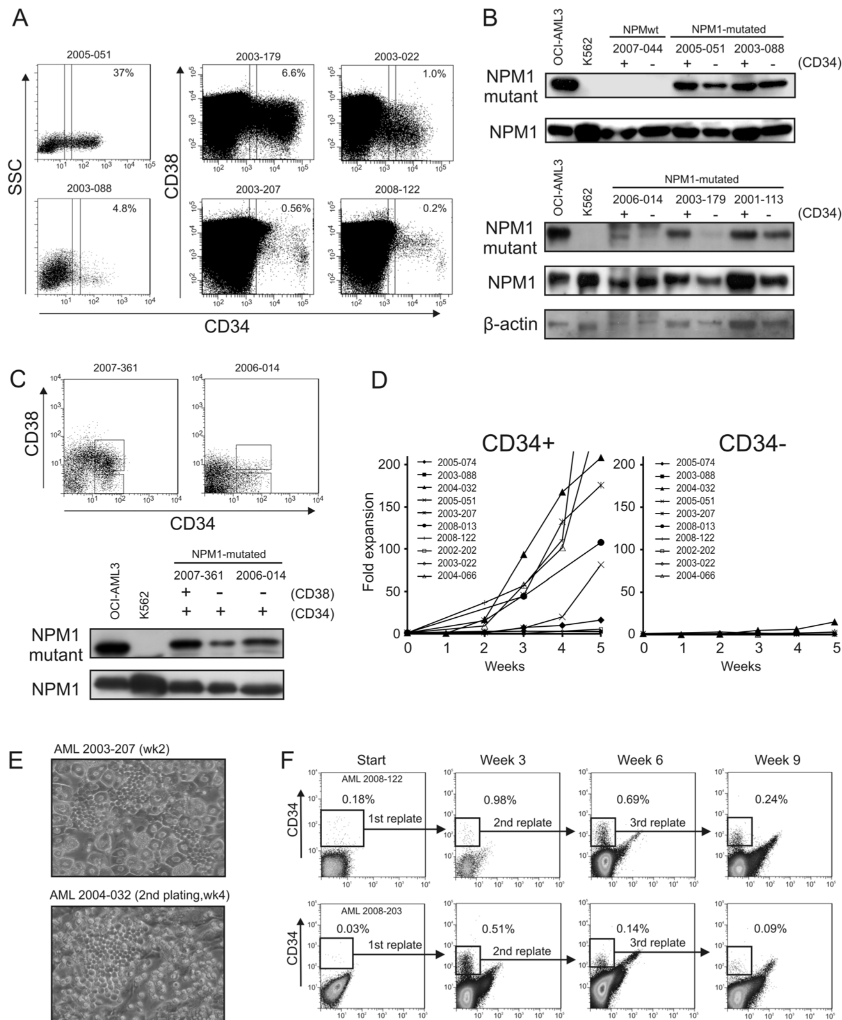

Figure 1 The CD34+ fraction of NPM1-mutated AML is part of the leukemic clone and is capable of long-term *ex vivo* expansion

(A) Flowcytometry experiments of six representative NPM1-mutated AML samples analyzing the CD34 expression. Percentages indicate the percentages of CD34+ cells that were sorted. (B) Western blot showing the presence of mutant NPM1 protein in both the CD34+ and CD34- cell fractions of NPM1-mutated AML cells. Cell lines K562 and OCI-AML3 were used as negative respectively positive control and NPM1 wild type and AML 2007-044 as a negative AML control for mutant NPM1. NPMwt = NPM1 wild type. (C) Western blot showing the presence of mutant NPM1 protein in both the CD34+/CD38+ and CD34+/CD38- cell fractions of NPMc+ AML cells. (D) Expansion of CD34+ cell fraction of NPM1-mutated AML on MS5 stromal layer. No expanding cultures could be established with CD34- NPM1-mutated AML cells. (E) Cobblestone areas, indicating cells under the stromal layer, starting within three weeks after initiating culture. After the second replating of cells initially started from CD34+ NPM1-mutated AML cells, again cobblestone areas were formed, indicating *ex vivo* self-renewal of the NPMc+ AML cells. Pictures of stromal co-cultures were taken with a 40x/0.60 objective. (F) Serial replating experiments of two representative NPM1-mutated AML samples are shown. Sorted CD34+ NPM1-mutated AML cells gave rise to CD34- progeny and CD34 percentages in the cultures dropped to very low percentages, comparable with the original CD34 percentage.

Since our data indicated that *in vitro* long-term self-renewing cultures could only be established by the $CD34^+$ $NPMc^+$ population, we questioned whether this relatively small $CD34^+$ population within $NPMc^+$ AML could be characterized by a specific gene expression profile. Patient characteristics of the AML samples used for this gene expression analysis are given in Supplemental Table 2. First, gene expression profiles of $CD34^+$ $NPMc^+$ AML samples (n=10) were compared with those of $CD34^+$ NPM1wt AML samples (n=33) and $CD34^+$ normal bone marrow (NBM) cells (n=24).[10] This comparison resulted in the identification of 267 differentially expressed probesets with a p-value $<1E10^{-6}$ (Supplemental Table 3). A supervised cluster analysis is shown in Figure 2A. Out of 267 genes, 88 were downregulated and 179 were upregulated in the $NPMc^+$ $CD34^+$ group. Gene ontology analysis revealed that the upregulated gene list was strongly and significantly enriched for homeobox genes of the A and B clusters. The cofactors MEIS1 and PBX3 were also amongst the upregulated genes, as well as the transcriptional regulators MSI2, JUND, GFI1 and RUNX1.
Secondly, we analyzed the differences in gene expression between $CD34^+$ (n=10) and $CD34^-$ (n=9) $NPMc^+$ AML cells. This comparison revealed 183 differentially expressed probesets with a p-value $<1E10^{-3}$ (Supplemental Table 4). A supervised cluster analysis is shown in Figure 2B. Interestingly, among the genes up-regulated in the $CD34^+$ $NPMc^+$ cells, some genes (like CD109, DCUN1D2, IPO11, SSH3) overlapped with genes identified as differentially expressed by human HSC and AML leukemic stem cells (LSC).[11]
Remarkably, we observed that the strong upregulation of HOX genes and MEIS1 was not only present in the self-renewing $CD34^+$ fraction, but also in the non-expanding $CD34^-$ fraction of $NPMc^+$ AML. Although the HOX genes and MEIS1 were consistently higher expressed in $NPMc^+$ $CD34^+$ cells compared to $NPMc^+$ $CD34^-$ cells, these differences did not reach significance (Figure 2C). The increased expression of MEIS1, HOXA9 and HOXB5 in $NPMc^+$ AML compared to NPM1wt AML was confirmed by qRT-PCR (Figure 2D).

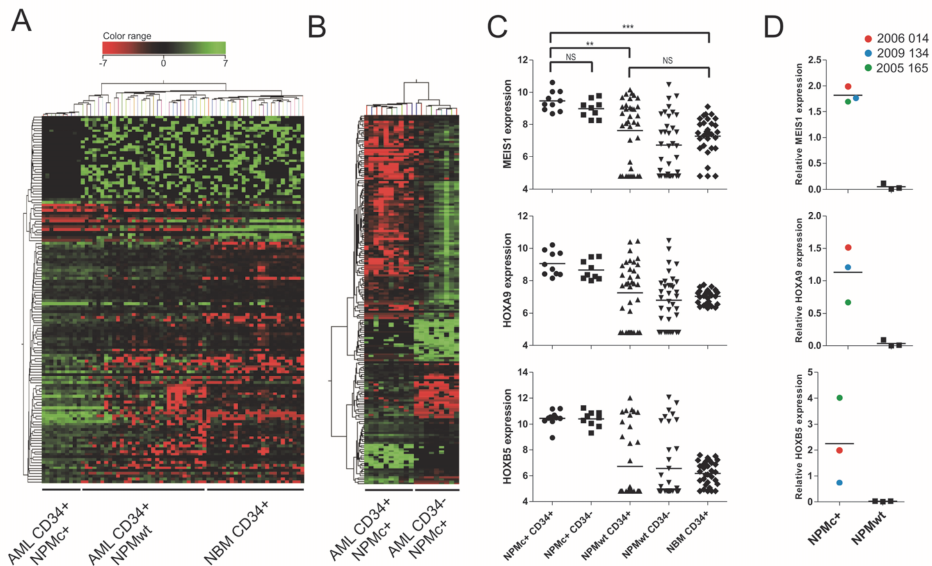

Figure 2 Gene expression profiling of CD34+ NPM1-mutated AML cells

(A) Supervised cluster analysis of AML CD34+ NPM1-mutated (NPMc+) (n=10) versus AML CD34+ NPMwt (n=33) and NBM CD34+ (n=24) (unpaired T-test, $p<1E10^{-6}$, 267 probesets). (B) Supervised cluster analysis of AML NPMc+ CD34+ (n=10) versus AML NPMc+ CD34- (n=9) (unpaired T-test, $p<1E10^{-3}$, 183 probesets). (C) Expression of MEIS1, HOXA9 and HOXB5 in CD34+ NPMc+, CD34- NPMc+, CD34+ NPMwt, CD34- NPMwt and CD34+ NBM samples. NS=not significant, **=p<0.01, ***=p<0.001. (D) The increased expression of MEIS1, HOXA9 and HOXB5 in NPMc+ AML compared to NPMwt AML was confirmed by real-time qRT-PCR. The colored dots indicate the AML IDs (indicated in the legend), two of these AMLs were used in the experiments shown in Figure 3C.

The observation that the expression of HOX genes and MEIS1 was also elevated within the CD34- NPMc+ compartment prompted us to determine whether HOXA/MEIS1 signaling would be required at all for the expansion of NPMc+ CD34+ AML cells. To address this question, downregulation of MEIS1 was achieved via lentiviral transduction of a short hairpin targeting MEIS1 (shMEIS1).[12] Downregulation of MEIS1 expression was confirmed by qRT-PCR as well as Western blotting (Figure 3A). Downregulation of MEIS1 in the NPMc+ AML cell line OCI-AML3 induced a strong growth reduction in these cells compared to cells transduced with a control scrambled short hairpin (shSCR) (Figure 3B). The reduced growth could at least partly be explained by increased apoptosis as examined by Annexin V/PI-staining (Supplemental Figure 1A). Assessment of differentiation and cell cycle demonstrated no distinct differences between shSCR and shMEIS1 transduced OCI-AML3 cells (Supplemental Figure 1B and 1C). Moreover, the expression of mutant and total NPM1 was not changed upon downregulation of MEIS1 and there were no indications for the involvement of senescence (Supplemental Figure 1D and 1E). Importantly, shMEIS1 transduction had no effect on cell growth in the MEIS1-negative BaF3 cell line (Figure 3B), excluding non-specific effects of

shMEIS1 transduction, and only a minor effect in the NPM1 wild type KG1a cells (Figure 3B). To further examine the effect of MEIS1 downregulation in NPMc$^+$ AML, primary CD34$^+$ NPMc$^+$ AML samples were transduced with shMEIS1 and control shSCR and expansion was evaluated in MS5 co-cultures. Downregulation of MEIS1 resulted in a strong reduction in expansion of primary CD34$^+$ NPMc$^+$ AML cells (Figure 3C), strongly suggesting the requirement of MEIS1 expression for long-term expansion of CD34$^+$ NPMc$^+$ AML cells. No distinct differences were observed in the expression of CD11b and CD15 between shSCR and shMEIS1 transduced NPMc$^+$ AML cells (Figure 3D). The increase in apoptosis upon downregulation of MEIS1 could be confirmed in primary AML cells (Figure 3E).

In the present study, we demonstrate that the CD34$^+$, but not the CD34$^-$ cells of NPMc$^+$ are capable of long-term expansion and serial-replating in our *ex vivo* stromal co-cultures. The CD34$^+$ cells gave rise to CD34$^-$ leukemic progeny upon culturing and serial replating, while CD34$^-$ cells never gave rise to CD34$^+$ cells, suggesting that the CD34$^+$ cells are at the top of the hierarchy in NPMc$^+$ AML. In addition, our data clearly indicate that MEIS1 is an important factor in NPMc$^+$ AML that is required for the long-term expansion of CD34$^+$ NPMc$^+$ cells. Yet, MEIS1 appears not to be sufficient for long-term expansion of leukemic cells, since we also observed high MEIS1 expression in the non-self-renewing CD34$^-$ cells of NPMc$^+$ AML. Thus, the cellular context in which HOX/MEIS1 is overexpressed appears to be very important, and other relevant genes are most likely required as well in order to maintain the expanding capacity of the CD34$^+$ NPMc$^+$ AML cells. In this perspective, the potential new target genes of NPMc$^+$ AML which could be identified by specifically studying gene expression profiles in the CD34$^+$ NPMc$^+$ AML cells are of interest. Our future studies will be aimed at the elucidation of the specific role that the identified genes might fulfill in NPMc$^+$ leukemia.

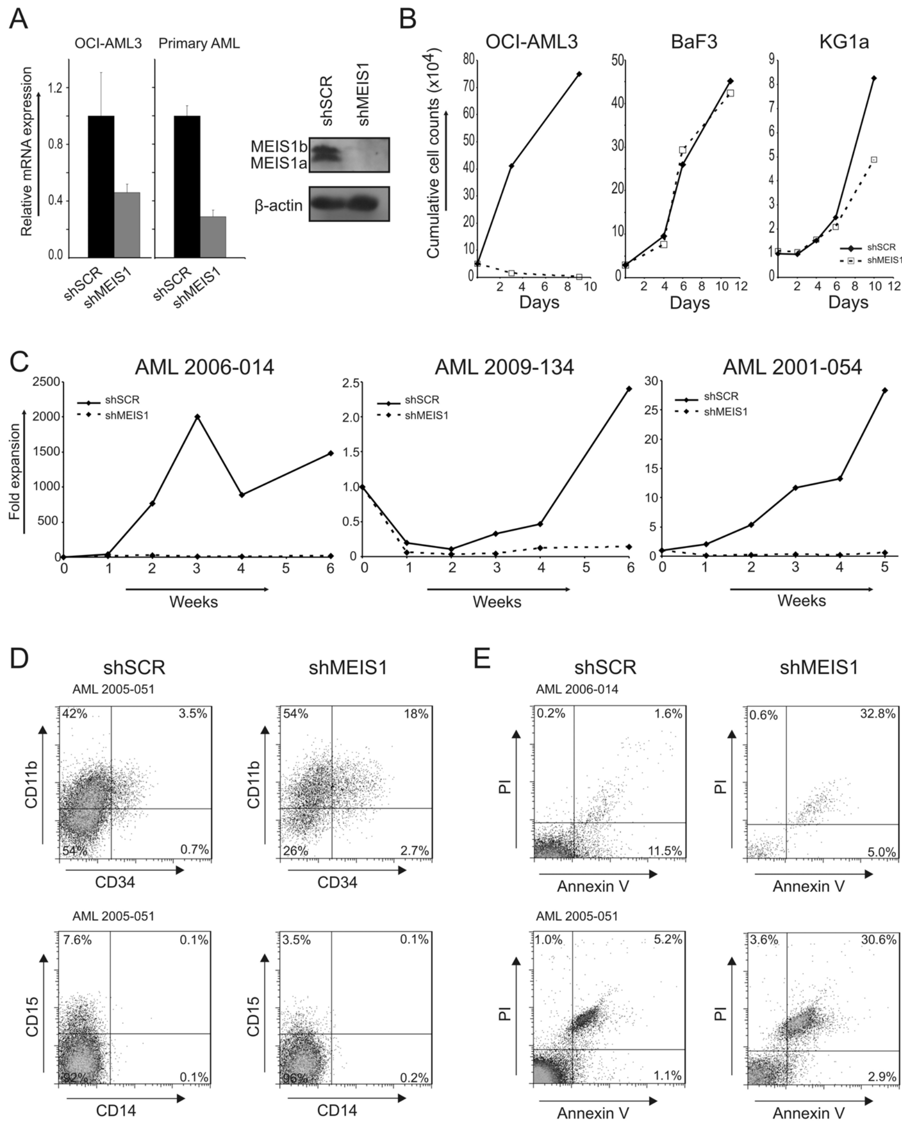

Figure 3 Downregulation of MEIS1 induces growth inhibition in NPM1-mutated AML

(A) The NPM1-mutated AML cell line OCI-AML3 and primary NPM1-mutated AML cells were transduced with a short hairpin against MEIS1 (shMEIS1) or scrambled control short hairpin (shSCR) and real-time qRT-PCR (left) was performed for MEIS1 using RPL27 and HPRT as housekeeping genes. For OCI-AML3 transduced cells also a western blot against MEIS1 (right) was performed. (B) Significant growth inhibition was seen in the NPM1-mutated AML cell line OCI-AML3 transduced with shMEIS1 compared to control shSCR transduction, while no effect on growth was seen in the transduced MEIS1-negative BaF3 cell line and only a minor effect in the NPM1 wild type cell line KG1a. (C) Transduction of primary CD34+ NPMc+ AML cells with shMEIS1 or control shSCR was performed and a significant growth inhibition was seen after downregulation of MEIS1 in all three primary NPMc+ AML samples. (D) No distinct differences were observed in the expression of CD11b and CD15 between shSCR and shMEIS1 transduced NPMc+ AML cells. (E) Downregulation of MEIS1 resulted in an increase in apoptosis in primary AML cells, as examined by Annexin V/PI staining.

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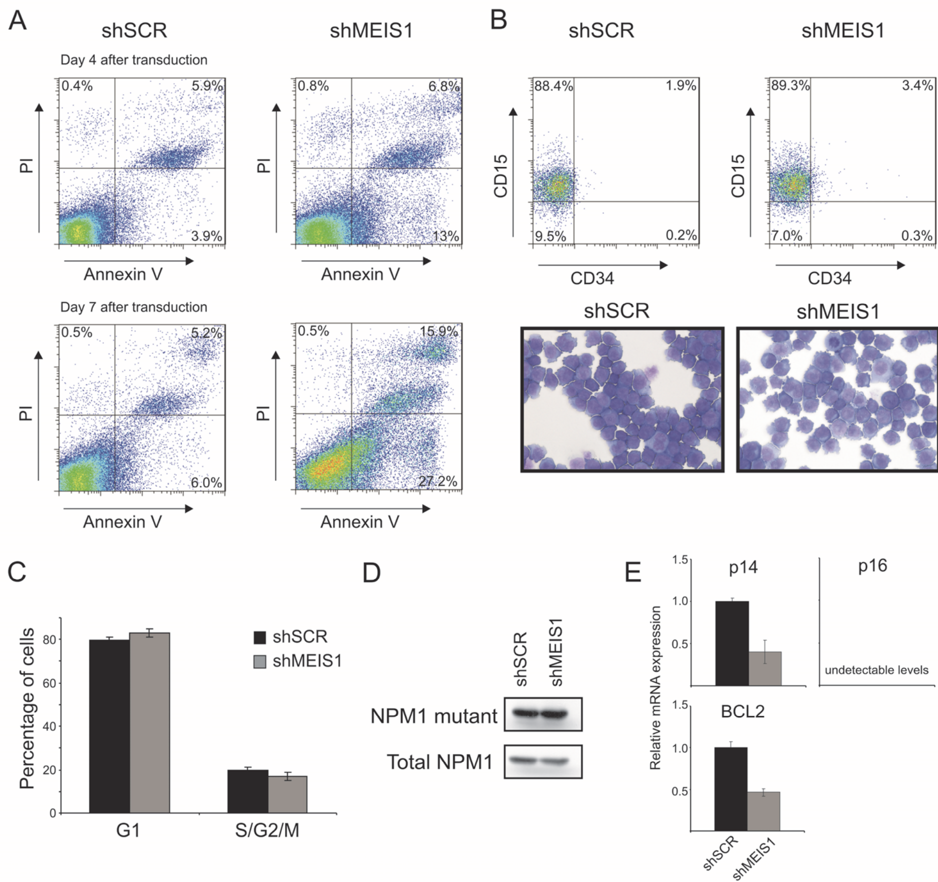
Supplemental Table 1 Characteristics of studied patient samples and performed experiments

Characteristics of patient samples						**Performed experiments**				
Patient ID	**BM/ PB**	**NPM1 (IHC)**	**CD34 (%)**	**FLT3**	**FAB**	**WB**	**Seq**	**MS5**	**CD34 replate**	**GEP**
2005-074	PB	nuc+cyt	0.02	wt	M0			x R		
1999-078	PB	nuc+cyt	11	ITD	M1					x
2002-202	PB	nuc+cyt	1.0	ITD	M1		+	x		
2006-014	PB	nuc+cyt	24	ITD	M1	+	+			x
2009-134	BM	nuc+cyt	14	ITD	M1		#			
2005-051	PB	nuc+cyt	37	ITD	M2	+	+	x R		x
2007-361	PB	nuc+cyt	22	wt	M2	#				
2008-203	PB	nuc+cyt	0.03	wt	M2				x	
2004-032	PB	nuc+cyt	2.0	ITD	M4		+	x R		x
2008-013	PB	nuc+cyt	0.19	wt	M4			x R		
2001-054	PB	nuc+cyt	11	ITD	M5		+			x
2001-113	BM	nuc+cyt	10	ITD	M5	+				x
2003-022	PB	nuc+cyt	1.0	ITD	M5		+	x		x
2003-088	BM	nuc+cyt	4.8	ITD	M5	+		x		x
2003-179	PB	nuc+cyt	6.6	ITD	M5	+			x	x
2003-207	BM	nuc+cyt	0.56	ITD	M5			x R		
2004-066	PB	nuc+cyt	1.0	ITD	M5			x R	x	
2005-165	PB	nuc+cyt	8.1	wt	M5		#			x
2008-122	PB	nuc+cyt	0.2	wt	M5		+	x R	x	
2007-44*	PB	nuc	49	ITD	M2	+				

BM = bone marrow; CD34 replate = serial replating experiment of sorted $CD34^+$ cells; cyt = cytoplasmic localization of NPM; IHC = immunohistochemistry; ITD = internal tandem duplication; MS5 = co-culture experiments on MS5 bone marrow cells; ND = not determined; NPM1 = nucleophosmin 1; nuc = nuclear localization of NPM; PB = peripheral blood; R = replated cells were capable of initiating 2nd expanding cultures; wt = wild type; WB = Western blot; Seq = sequencing of NPM1; GEP = gene expression profiling using Illumina BeadArrays; x = performed; + = performed and NPM1 mutation detected in both $CD34^+$ and $CD34^-$ subfraction. # = performed and NPM1 mutation detected in both $CD34^+/CD38^-$ and $CD34^+/CD38^+$ subfraction. All patient samples had a normal karyotype AML. * Control AML sample.

Other supplemental tables can be found at the Leukemia website.



Supplemental Figure 1 The effects of downregulation of MEIS1 in OCI-AML3 cells
(A) Downregulation of MEIS1 results in an increase in apoptosis as examined by Annexin V/PI staining. No distinct differences between shSCR and shMEIS1 transduced OCI-AML3 were found for (B) differentiation, as assessed by CD15 expression and MGG-staining, and (C) cell cycle analysis, as examined by Hoechst staining. (D) Downregulation of MEIS1 did not change the expression of NPM mutant or total NPM protein. (E) No upregulation of p14 or p16 expression was found by qRT-PCR. The increase in apoptosis was accompanied by a decrease in mRNA expression of BCL2 in shMEIS1 transduced OCI-AML3 cells.