MEIS1 regulates early erythroid and megakaryocytic cell fate

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ABSTRACT

MEIS1 is a transcription factor expressed in hematopoietic stem and progenitor cells and in mature megakaryocytes. This biphasic expression of *MEIS1* suggests that the function of MEIS1 in stem cells is distinct from its function in lineage committed cells. Mouse models show that Meis1 is required for renewal of stem cells, but the function of MEIS1 in human hematopoietic progenitor cells has not been investigated. We show that two *MEIS1* splice variants are expressed in hematopoietic progenitor cells. Constitutive expression of both variants directed human hematopoietic progenitors towards a megakaryocyte-erythrocyte progenitor fate. Ectopic expression of either *MEIS1* splice variant in common myeloid progenitor cells, and even in granulocyte-monocyte progenitors, resulted in increased erythroid differentiation at the expense of granulocyte and macrophage differentiation. Conversely, silencing *MEIS1* expression in progenitor cells induced a block in erythroid expansion and decreased megakaryocytic colony formation capacity. Gene expression profiling revealed that both *MEIS1* splice variants induce a transcriptional program enriched for erythroid and megakaryocytic genes. Our results indicate that *MEIS1* expression induces lineage commitment towards a megakaryocyte-erythroid progenitor cell fate in common myeloid progenitor cells through activation of genes that define a megakaryocyte-erythroid-specific gene expression program.

Introduction

Production of appropriate numbers of distinct blood cell types is dependent on controlling lineage commitment in hematopoietic tissues and terminal differentiation into circulating blood cells. Transcription factors regulate the renewal of hematopoietic stem cells (HSC) and specify distinct lineages.[1-9] By also controlling each other's expression, transcription factors induce lineage-specific gene expression and repress other differentiation routes. The differentiation of common myeloid progenitors (CMP) towards either granulocyte-monocyte progenitors (GMP) or megakaryocyte-erythrocyte progenitors (MEP), for example, is regulated by transcription factors, among which GATA1 plays a crucial role.[10,11] However, depletion of *Gata1* alone does not impair MEP differentiation or enhance myeloid output, indicating that additional factors regulate the megakaryocyte-erythroid fate.[12]

The *Meis1* (*myeloid ecotropoic viral insertion site 1*) transcription factor gene was first described as a common viral integration site associated with tumor formation in BXH-2 mice.[13] *MEIS1* overexpression together with overexpression of *HOXA9* sustains leukemic stem cell potential in human acute myeloid leukemia and childhood acute lymphoblastic leukemia.[14,15]

MEIS1 is a homeobox gene of the TALE (three amino acids loop extension) family, encoded on human chromosome 2p15. The *MEIS1* amino terminus contains binding domains for its interaction partners, the transcription factors *HOXA9* and *PBX1*, followed by a homeobox DNA binding domain and carboxy-terminal transcriptional activation domains.[16] Previous studies have shown that *MEIS1* pre-mRNA undergoes alternative splicing in a highly conserved manner resulting in four splice variants of which two, *MEIS1B* and *MEIS1D*, are expressed in hematopoietic cells.[13,17] MEIS1B and MEIS1D proteins differ in their carboxy termini, with MEIS1D being the transcriptionally more active splice variant.[18]

MEIS1 is expressed in hematopoietic stem and progenitor cells[9,19,20] and increases during human megakaryocytic differentiation.[21] *Meis1* null-mutant mice die at embryonic day 14.5 due to a poorly developed hematopoietic compartment, lack of megakaryocytes and platelets and defective vascularization.[6,7,22] *Meis1*-deficient zebrafish display a lack of megakaryocytes, decreased erythrocyte levels and vascularization defects,[23] consistent with the finding in null-mutant mice. *Meis1* depletion in murine HSC using lineage-specific Cre-recombinase results in cell cycle entry and loss of quiescence.[9]

The most prominent murine models used to study Meis1 function to date rely on homologous recombination resulting in Meis1 depletion during embryogenesis. Given the construction of these knockouts it was not possible to compare the function of the different Meis1 splice variants or specifically study MEIS1 in hematopoietic cells in these murine models.[6,7] Here, we investigated for the first time the role of *MEIS1* in adult human hematopoietic progenitor cells. Expression of the two MEIS1 splice variants was modulated in sorted hematopoietic stem and progenitor cell subsets using lentiviral knockdown or overexpression of MEIS1 fused to green fluorescent protein (GFP), an

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approach that had been described before,[24] enabling us to delineate the effects of MEIS1 on specific stages of lineage commitment. Furthermore, using transcriptional profiling of MEIS1-overexpressing CD34+ hematopoietic stem and progenitor cells, we generated an overview of the transcriptional changes following MEIS1 expression.

Methods

Additional information is provided in the *Online Supplement*.

Human CD34+ cells

Mobilized peripheral blood (MPB) and cord blood (CB) cells were obtained after informed consent and with the approval of the ethical committee of local hospitals. Bone marrow was collected by sternal puncture from patients undergoing cardiac surgery. CD34+ cells were isolated from CB or MPB samples within 48 hours after arrival of the material using magnetic cell sorting according to the manufacturer's specifications (Miltenyi Biotech, Bergisch Gladbach, Germany). All samples contained more than 90% CD34+ cells and less than 5% CD41 determined by flow cytometry (LSRII, Becton Dickinson).

Lentiviral transduction, colony formation assays and human primary cell culture

CD34+ cells were transduced with MEIS1 expression or shRNA constructs and incubated overnight in CellGro medium (CellGenix, Frankfurt, Germany) with 100 ng/mL each of stem cell factor (SCF, R&D Systems Abingdon, UK), thrombopoietin (TPO; Sanquin PeliKines, Amsterdam, The Netherlands), Fms-like tyrosine kinase3-ligand (Flt3; Miltenyi Biotech) and 20 ng/mL interleukin 6 (IL6; Miltenyi Biotech). Forty-eight hours after transduction, cells were sorted for GFP-expression using the FACSAria II Cell Sorter (BD Biosciences). GFP-positive cells were used in either Colony Gel colony formation assays (Cell Systems, Frankfurt, Germany) or Megacult colony formation assays (Stem Cell Technologies, Grenoble, France), according to the manufacturers' specifications. Erythroid differentiation in liquid culture was achieved by seeding CD34+ cells into CellGro medium (CellGenix) with 100 ng/mL of SCF (R&D Systems), 3 U/mL erythropoietin (EPO; R&D Systems) and 10 ng/mL interleukin 3 (IL-3; R&D Systems). For transduction of committed erythroid progenitor cells, CD34+ cells were cultured towards the erythroid lineage in CellGro medium (CellGenix) as described above. After 7 days in culture, expression of CD71 (transferrin receptor) and CD235a (glycophorin A) was measured using flow cytometry (LSRII, Becton Dickinson). Cells were transduced with MEIS1 expression or knockdown constructs and left to differentiate towards erythroid cells for a further 5 days. At the end of the culture cells were counted, labeled with PeCy7-conjugated CD34 (BD Biosciences) and tricolor-conjugated CD235a (Caltag Laboratories, Buckingham, UK) and analyzed using flow cytometry (LSRII, Becton Dickinson).

Hematopoietic stem and progenitor cell subfractions

CD34+cells from MPB samples were stained with PE-Cy7-conjugated CD34, PerCP-conjugated CD38, FITC-conjugated CD45RA, V450 Horizon-conjugated CD45, APC-conjugated BAH clone recognizing a MEP-specific epitope and PE-conjugated CD123 (all from BD Biosciences). Cells were sorted into HSC (CD34+/CD38-), CMP (CD34+/CD38+/CD123+/CD45RA-), GMP (CD34+/CD38+/CD123+/CD45RA^{dim}) and MEP (CD34+/ CD38+/ CD123-/CD45RA-/BAH1+)[25,26] using a FACS Aria II cell sorter (BD Biosciences). After sorting, subfractions were transduced with virus particles carrying MEIS1B or MEIS1D expression constructs or an empty vector control. Forty-eight hours after transduction, cells were sorted for GFP-expression and 1000 GFP-positive cells were seeded into Colony Gel for the colony formation assay (Cell Systems) according to the manufacturer's specifications.

Results

Differential expression of MEIS1 splice variants in human CD34+ hematopoietic stem and progenitor cells

To investigate the expression of the *MEIS1B* and *MEIS1D* splice variants (Figure 1A) in human primary hematopoietic stem and progenitor cells, we sorted CD34+ cells into subfractions to obtain HSC, CMP, GMP and MEP.[25,26] *MEIS1* mRNA was present in all subfractions, with the expression of *MEIS1D* being 4-fold higher than *MEIS1B* (Figure 1B). Expression of both *MEIS1* splice variants decreased when HSC differentiated towards the more committed CMP, GMP, and MEP (Figure 1B).

When CD34+ cells from MPB or CB were allowed to differentiate into megakaryocytes, MEIS1D protein expression decreased while mRNA was still present. MEIS1B mRNA and protein expression increased throughout megakaryopoiesis, with expression being highest in terminally differentiated megakaryocytes (Figure 1C,D). Taken together, our data show that MEIS1B and MEIS1D are present in hematopoietic stem cells and their expression decreases when the cells differentiate into more committed progenitors. Expression of MEIS1 is then reinforced during megakaryopoiesis with MEIS1B being the more abundant splice variant.

MEIS1 expression skews CD34+ cells towards a megakaryocyte-erythrocyte progenitor fate

To investigate the functional role of *MEIS1* during lineage commitment of hematopoietic stem and progenitor cells, we ectopically expressed *MEIS1* in CD34+ cells using lentiviral expression vectors with *MEIS1B* or *MEIS1D* full length cDNA fused to GFP at the amino-terminus. The functionality of the fusion protein has already been reported.[24] *MEIS1B* expression increased 60-fold in sorted GFP-positive cells 48 h after transduction, whereas that of *MEIS1D* increased 15-fold compared to empty vector controls (Figure 2A), expression levels well within the range of physiological *MEIS1* fluctuation. Seeding transduced CD34+ cells one cell per well in semisolid media promoting erythroid or granulocyte-macrophage differentiation did not alter total colony numbers (empty vector 16±6.5, *MEIS1B* 24±13.5, *MEIS1D* 16±13.8) while the phenotypic distribution significantly changed. The numbers of burst-forming unit erythroid (BFU-E) and colony-forming unit erythroid (CFU-E) increased by 3-fold with *MEIS1B* overexpression and 1.9-fold with *MEIS1D* compared to empty vector. Colony-forming unit granulocyte-macrophage (CFU-GM) numbers decreased by 3-fold with *MEIS1B* and 6-fold upon *MEIS1D* expression compared to empty vector (Figure 2C).

Next, expression of both *MEIS1* variants was silenced in CD34+ cells with short hairpins targeting either exon 7 (sh72) or the 3' UTR (sh68), sequences present in both *MEIS1* variants (Figure 2B) reducing *MEIS1B* expression by 60% with sh68 and about 70% with sh72. When transduced CD34+ cells were plated out in semisolid media, *MEIS1* knockdown resulted in significantly less total colony formation with sh72 (4±1.8, $P<0.01$) than with either the

short hairpin control shc002 (25±2.5) or sh68 (19±2.6). Furthermore, knockdown almost completely abrogated erythroid colony formation with sh68 or sh72 compared to shc002 (Figure 2D). CFU-GM numbers were not affected by *MEIS1* knockdown (Figure 2D) suggesting that *MEIS1* expression controls erythroid *versus* granulocytic differentiation. In parallel, transduced CD34⁺ cells were plated in semi-solid media promoting megakaryocytic differentiation. *MEIS1B* and *MEIS1D* overexpression increased the number of megakaryocytic colonies by 1.6-fold (174±81) and 2-fold (237±57), respectively, compared to the vector control (111 ± 16) (Figure 2E). In addition to increased colony numbers, the colony size was larger (Figure 2F) upon overexpression with either one of the two *MEIS1* splice variants. *MEIS1* knockdown in CD34⁺ cells reduced megakaryocytic colony formation by 18% with sh68 (152±38) and by 30% with

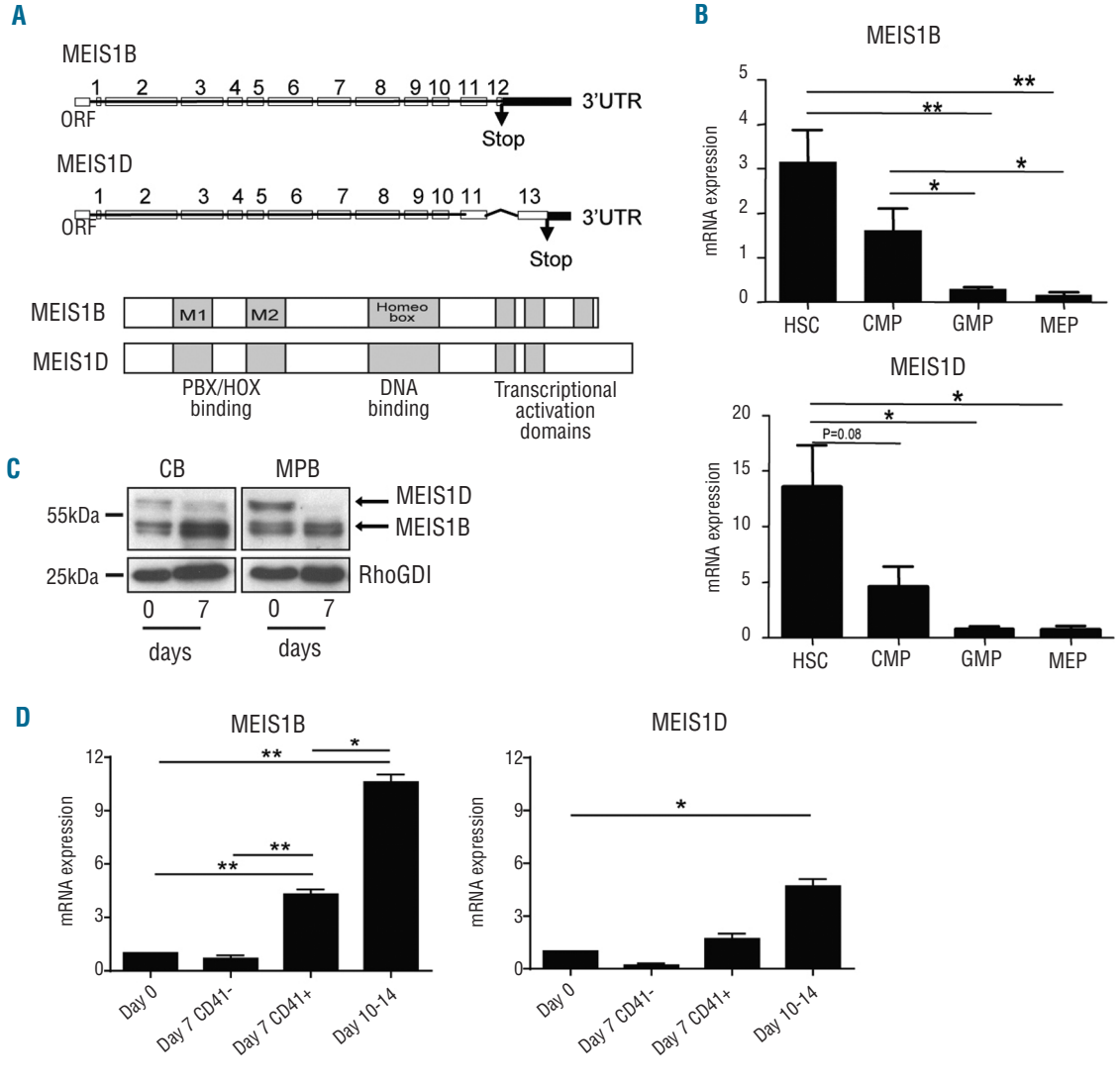

Figure 1. ***MEIS1*** **splice variants are differentially expressed in hematopoietic stem and progenitor cells. (A) Schematic view of** ***MEIS1B*** **and** ***MEIS1D*** **mRNA sequences (upper two) and functional protein domains (lower two). Numbered boxes indicate exons. ORF: open reading frame; M1, M2: binding domains for trimerization with PBX1/HOXA9 proteins; homeobox: DNA binding domain. (B)** ***MEIS1B*** **and** ***MEIS1D*** **expression in bone marrow CD34⁺ cells sorted into hematopoietic stem and progenitor cell subsets. Bone marrow-derived CD34⁺ cells were enriched for hematopoietic stem cells (HSC), common myeloid progenitors (CMP), granulocyte-monocyte protenitors (GMP) and megakaryocyte-erythroid progenitors (MEP) using flow cytometry followed by RNA isolation. cDNA was synthesized and quantitative real-time polymerase chain reaction (q-RT-PCR) was performed with specific primers for** ***MEIS1B*** **or** ***MEIS1D***. **Gene expression was calculated relative to the β-glucoronidase (*GUS*) reference gene using the deltaCT method. Experiments were performed in triplicate with CD34⁺ cells from three independent donors and the data were analyzed using an unpaired two-tailed Student t-test. (C) Western blot analysis of MEIS1 expression in CB- and MPB-derived CD34⁺ cells directly after isolation (day 0) and after 7 days in culture promoting megakaryopoiesis with TPO and IL-1β. RhoGDI was used to show equal protein loading. A representative image is shown from three biologically independent experiments. (D)** ***MEIS1B*** **and** ***MEIS1D*** **mRNA during megakaryopoiesis. RNA samples were taken from MPB-derived CD34⁺ cells, which were then cultured towards megakaryocytes. Additional RNA samples were taken at day 7 of megakaryocyte culture from sorted CD41⁺ and CD41⁻ fractions. CD41⁺ cells were cultured further until day 14 and a last RNA sample was taken. cDNA was generated and** ***MEIS1*** **expression was determined using qRT-PCR. Data from three biologically independent experiments are shown. ****P*<0.05, *****P*<0.01.

sh72 (145±39) (Figure 2G) and colony size compared to shc002 (174±32) (Figure 2H). These results suggest that *MEIS1* expression, especially *MEIS1D*, induces a MEP fate in hematopoietic stem and progenitor cells.

MEIS1 *induces erythroid commitment in myeloid progenitor cells*

To determine at which level of hematopoietic commitment the *MEIS1* variants exert their effects, MPB-derived CD34⁺ cells were sorted into HSC, CMP and GMP (Figure 3A), transduced with *MEIS1B* or *MEIS1D* overexpression vectors, sorted for GFP expression and seeded into semisolid medium. Neither *MEIS1B* nor *MEIS1D* overexpression significantly changed the differentiation potential of the HSC-enriched fraction (Figure 3B). Expression of either splice variant in the CMP caused a 2-fold increase in erythroid colonies, accompanied by a significant decrease in CFU-GM (Figure 3C) reflecting the phenotype of total CD34⁺ cells after *MEIS1* overexpression (Figure 2C). Strikingly, expression of the MEIS1 splice variants enabled the formation of erythroid colonies in the GMP fraction, a progenitor subset committed to differentiate into granulocytic-monocytic cells. The increase in erythroid colonies was accompanied by a significant reduction in CFU-GM (Figure 3D), suggesting that *MEIS1* is able to reprogram GMP towards erythroid commitment. Our findings show

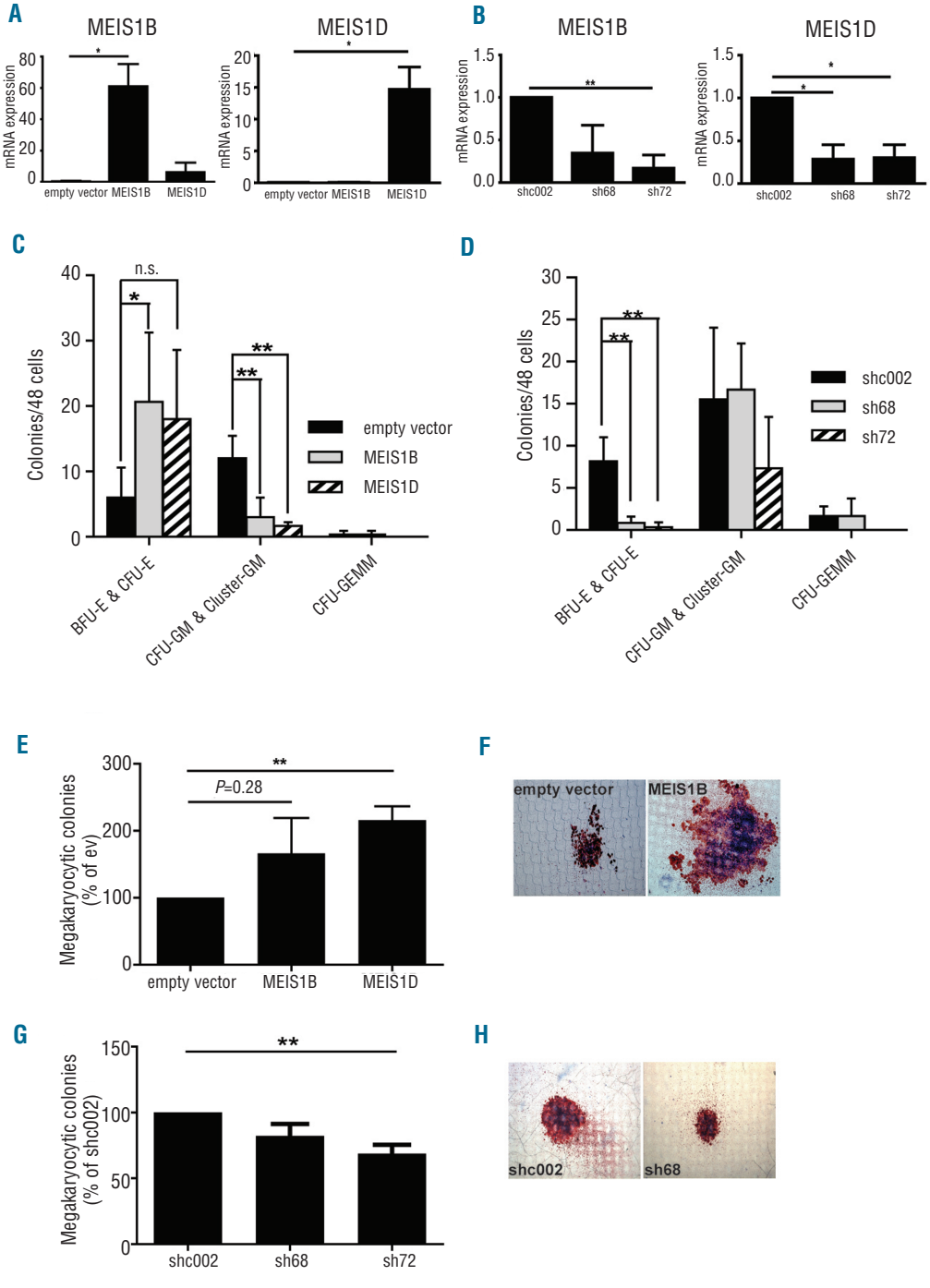

Figure 2. ***MEIS1*** **expression skews CMP towards a MEP-fate. (A-B) Lentiviral transduction of MPB-derived CD34⁺ cells with** ***MEIS1*** **overexpression constructs encoding** ***MEIS1B*** **or** ***MEIS1D*** **cDNA N-terminally fused to GFP, or with shRNA (sh68, sh72) targeting both** ***MEIS1*** **variants. Forty-eight hours after transduction, cells were sorted using GFP expression, and lysed for RNA isolation.** ***MEIS1*** **expression was calculated relative to β-glucoronidase. (C-D) Colony formation assay from MPB-derived CD34⁺ cells transduced with** ***MEIS1*** **overexpression constructs and colony formation assay after** ***MEIS1*** **knockdown in CB CD34⁺ cells. Transduced cells were plated (single cell/single well) into 48 wells of a 96-well plate. (E-H) Megacult colony formation assay using transduced MPB-derived CD34⁺ cells. Transduced, GFP-positive cells (2,500) were seeded into semisolid medium. After 2 weeks, colonies were fixed, stained for CD41 expression (red) and Evans Blue as counterstain (blue) and CD41⁺ colonies were counted. Representative images were taken with a bright-field microscope using a 10x magnification. All experiments were performed in triplicate using cells from three independent donors. Statistical analysis was carried out using an unpaired two-tailed Student t-test. *P<0.05, **P<0.01.**

that expression of *MEIS1B* and *MEIS1D* skews CMP towards a MEP fate and that *MEIS1* expression reprograms GMP into the erythroid lineage.

MEIS1 *positively regulates erythroid determination*

To further examine the role of MEIS1 in erythroid commitment and differentiation, transduced CD34$^+$ cells were grown in liquid culture supporting erythroid proliferation and differentiation.

A 2.3-fold increase in cellular yield was observed upon overexpression of *MEIS1B* and a 2.2-fold increase was found with *MEIS1D* overexpression compared to empty vector controls (Figure 4A). While cell numbers increased with *MEIS1* expression, glycophorin A (CD235a) expression was the same for *MEIS1B*- or *MEIS1D*-expressing cells as well as for empty vector cells (Figure 4A) suggesting that *MEIS1* induces skewing of CD34$^+$ cells towards an erythroid fate, but not erythoid differentiation. *Vice versa*, *MEIS1* knockdown caused a 47% reduction in cell numbers with sh68 and a 60% reduction with sh72 compared to control cells (Figure 4B). The decline in erythroid cells was accompanied by a 25% decrease in CD235a expression with sh68 and a 57% decrease with sh72 (Figure 4B), further underlining that *MEIS1* expression is needed to direct CD34$^+$ progenitor cells to an erythroid fate.

To determine whether *MEIS1* also affects committed erythroblasts, CD34$^+$ cells were first cultured for 7 days to establish an erythroid fate, and subsequently transduced with *MEIS1* overexpression and knockdown constructs. After an additional 4 days of culture, overexpression of *MEIS1B* or *MEIS1D* resulted in 2-fold more cells compared to empty vector (Figure 4C). A trend towards increased CD235a expression was also detected (Figure 4C), but differences did not reach statistical significance. Silencing *MEIS1* did not affect the amount of cells (Figure 4D), consistent with a physiological downregulation of *MEIS1B* and *MEIS1D* during physiological erythropoiesis (Figure 4E). Together these findings indicate that MEIS1 expression is crucial to establish an erythroid fate in hematopoietic progenitor cells but is not needed for erythoid differentiation.

MEIS1 *expression induces a megakaryocyte-erythroid progenitor fate*

Elevated *MEIS1B* or *MEIS1D* expression increased ery-

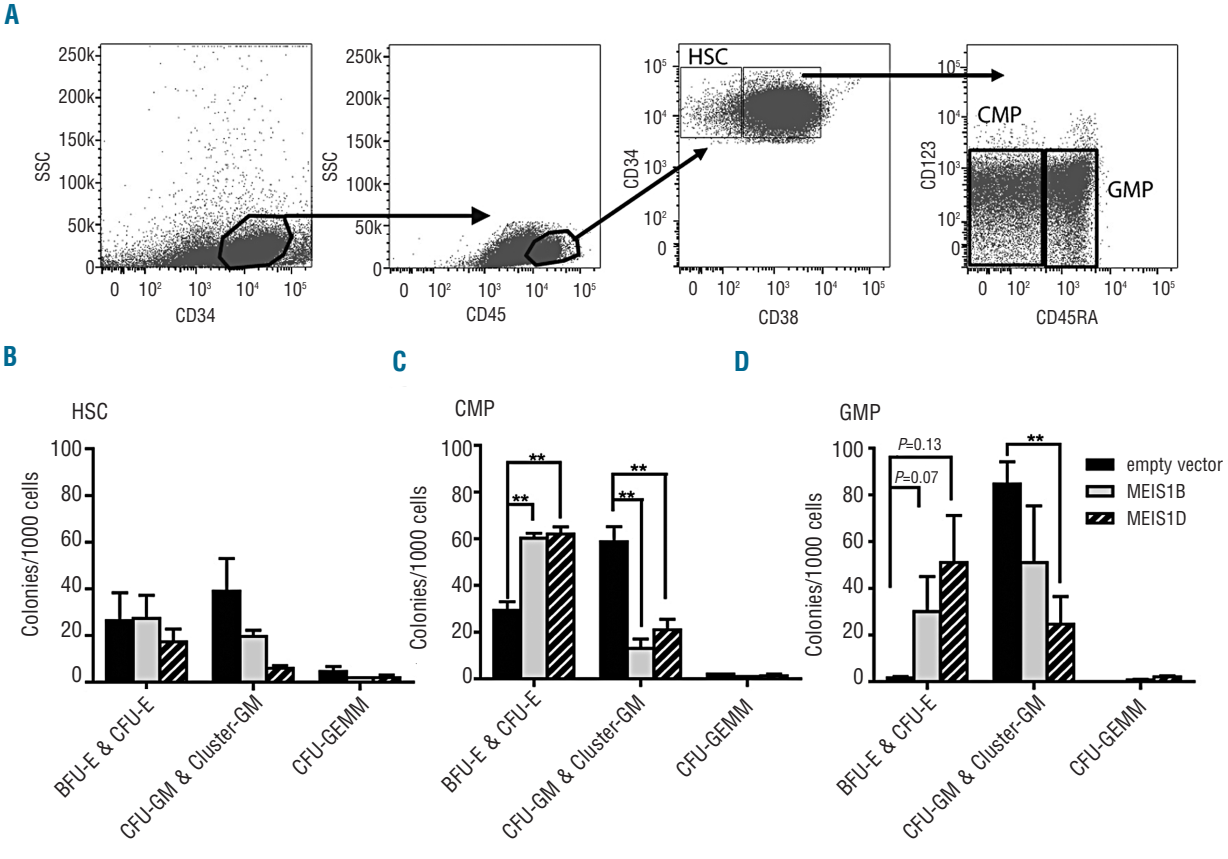

Figure 3. MEIS1 induces erythroid commitment in myeloid progenitor cells (A) Sorting of MPB-derived CD34$^+$ cells for HSPC. CD34$^+$/CD38$^-$ indicates HSC, CMP are characterized by a CD34$^+$/CD38$^+$/CD123$^-$/CD45RA$^-$ phenotype and GMP are defined as CD34$^+$/CD38$^+$/CD123$^+$/CD45RA^{dim}. Colony formation assay of sorted (B) HSC, (C) CMP and (D) GMP after *MEIS1* overexpression. CD34$^+$ cells were sorted into stem and progenitor subsets, transduced with *MEIS1* overexpression constructs, and 48 h after transduction sorted again for GFP expression. One thousand GFP-positive cells per condition were seeded into semisolid medium, and colonies were counted after 2 weeks of incubation. Experiments were performed in triplicate with CD34$^+$ cells from three independent donors. Statistical analysis was carried out using an unpaired two-tailed Student t-test. **P*<0.05, ***P*<0.01.

throid colony formation from CMP and GMP, suggesting that *MEIS1* induces a MEP gene expression profile in these progenitors. To characterize the transcriptional changes caused by *MEIS1*, the gene expression profile of CD34$^+$ cells from MPB was determined 48 h after transduction with GFP-fused *MEIS1B* or *MEIS1D* expression vectors, or the empty vector control. Total RNA from three biologically independent experiments was hybridized to an Illumina Human HT-12 v4 expression bead array. After VSN normalization, gene expression was compared between the three different groups (empty vector, *MEIS1B*, or *MEIS1D*) using Benjamini Hochberg FDR values smaller than 0.05 as a cut-off. Sixty-six genes were differentially regulated upon *MEIS1B* overexpression, 125 genes with *MEIS1D* overexpression and 69 genes were differentially regulated in both overexpression conditions compared to empty vector controls. Overexpression of either *MEIS1B* or *MEIS1D* resulted in similar expression patterns, indicating that both splice variants regulate common targets (Figure 5A, Table 1, *Online Supplementary Table S1*). The *MEIS1*-regulated genes in

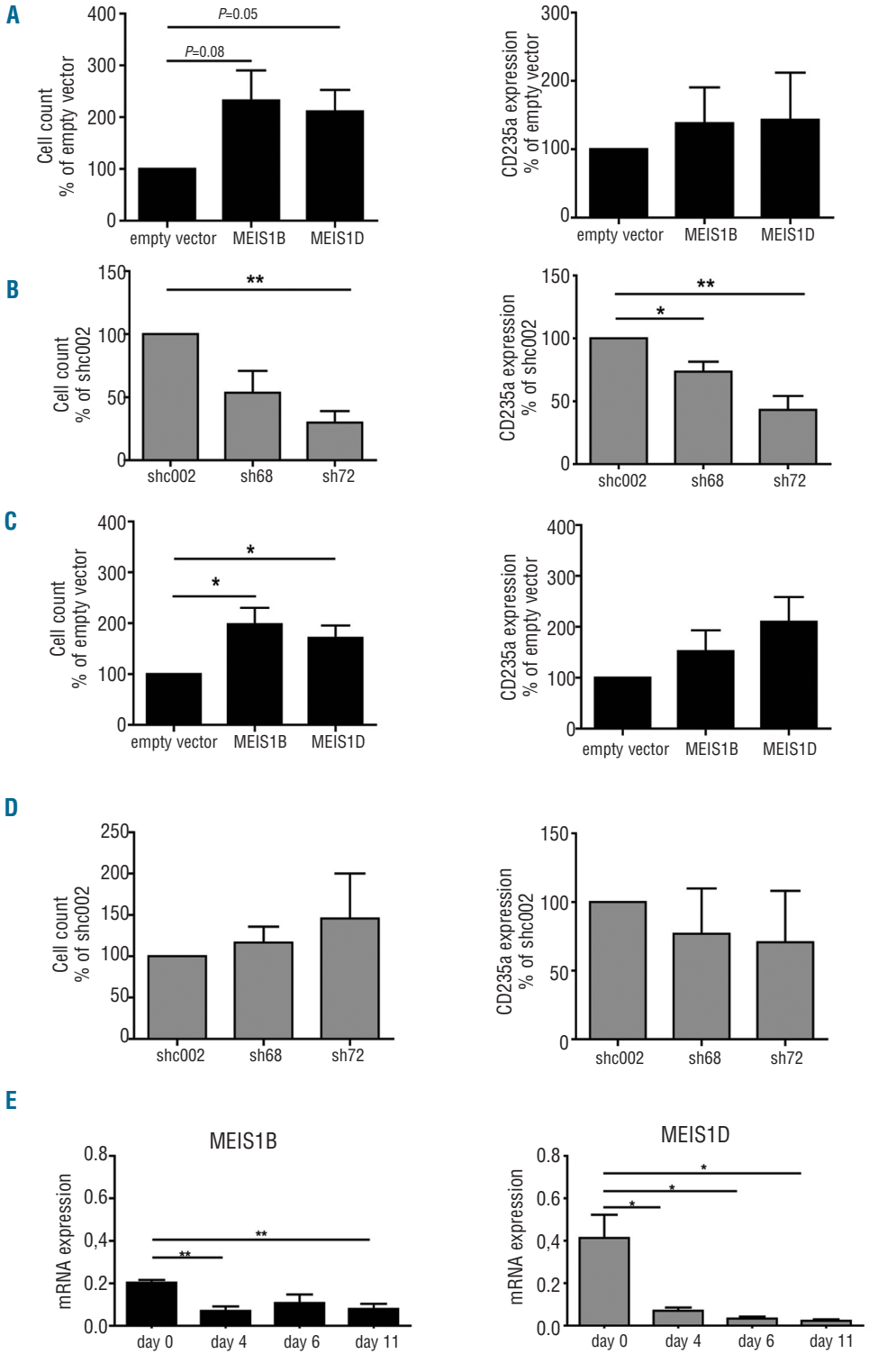

Figure 4. *MEIS1* positively regulates erythroid determination. (A, B) Lentiviral transduction of MPB-derived CD34$^+$ cells with *MEIS1* overexpression constructs, and subsequent erythroid differentiation. Transduced cells were allowed to differentiate for 11 days in the presence of EPO, SCF, and IL-3. After 11 days in culture, cell counts and glycophorin A (CD235a) expression were determined with flow cytometry using counting beads. Cell count and CD235a expression after (A) *MEIS1* overexpression or (B) *MEIS1* knockdown. (C, D) Lentiviral transduction of committed erythoid cells. CD34$^+$ cells were allowed to differentiate for 7 days in the presence of EPO, SCF, and IL-3. After 7 days in culture, cells were transduced with *MEIS1B* or *MEIS1D* and left to differentiate for an additional 5 days. At the end of the culture, cell counts and glycophorin A (CD235a) expression were determined. (C) Transduction of erythoid cells with *MEIS1* overexpression constructs (D) Lentiviral transduction of erythroid cells with *MEIS1* shRNA constructs. Cell count was determined as described in (C). (E) Real-time PCR of *MEIS1B* and *MEIS1D* expression during erythroid differentiation. At the beginning of the culture as well as after 4, 6 and 11 days, RNA samples were taken for expression profiling. *MEIS1* expression was calculated relative to *GUS*. Experiments were performed in triplicate from three independent donors. Statistical analysis was carried out using an unpaired two-tailed Student t-test. **P*<0.05, ***P*<0.01.

Figure 5. *MEIS1* expression maintains a progenitor cell fate thus enabling multilineage differentiation. (A) Heatmap showing gene expression profiles in CD34⁺ cells 48 h after transduction with *MEIS1B* or *MEIS1D* overexpression vectors from three independent experiments. (B) Comparison of gene expression between Haematlas lineage-specific genes and microarray on CD34⁺ cells after *MEIS1* overexpression: percentage gene overlap with Haematlas lineage-specific genes set at 100% and number of overlapping genes from microarray calculated relative to Haematlas.

CD34⁺ cells included transcripts whose expression had previously been described to be regulated by *MEIS1*, such as cyclin D1 (*CCND1*)[9,24] and cyclin D3 (*CCND3*)[9,27] (Table 1, *Online Supplementary Table S1*).

Together with these previously described targets, *MEIS1* increased expression of genes associated with megakaryopoiesis and erythropoiesis such as *KLF1*, *HBD*, *HBG*, *SLC40A1*, *THBS1*, *GPIb*, *VWA5A* and *GATA2* (Table 1, *Online Supplementary Table S1*). Notably, transcripts known to be specific for HSC were downregulated upon *MEIS1* expression, including *PROM1* (CD133), *AK2* and *CD34*. *CD48*, a gene marking granulocytic cell commitment, was also downregulated.

To examine the lineage specification induced upon *MEIS1* expression further, we compared our expression profiling data with the Haematlas.[21] In this study, genes specific for CD4⁺ T helper lymphocytes, CD8⁺ T cytotoxic lymphocytes, CD14⁺ monocytes, CD19⁺ B lymphocytes, CD56⁺ natural killer cells and CD66⁺ granulocytes were identified based on expression profiling. The percentage of common genes was most pronounced between *MEIS1*-overexpressing CD34⁺ cells and myelo-erythroid lineage cells, resulting in 30% (erythroblasts) and 38% (megakaryocytes) common transcripts (Figure 5B, Table 2 and *Online Supplementary Table S2*). *Vice versa*, few gene expression patterns were shared between *MEIS1*-overexpressing CD34⁺ cells and cells of the lymphoid lineage with 10.8% in CD56⁺ natural killer cells and a maximum of 14.6% genes expressed in CD19⁺ B lymphocytes. The increase in the amount of overlapping expression profiles underlines that *MEIS1* overexpression in CD34⁺ cells induces an expression profile characteristic of megakaryocytes and erythroid cells.

To characterize the role of *MEIS1* on lineage determination, gene set enrichment analysis was performed. In a previous study, Jaatinen *et al.* compared the expression profiles of CB-derived CD133⁺ HSC and CD133⁻ progenitor cells.[28] When the expression profile of CD34⁺ cells overexpressing *MEIS1* was compared with that of CD133 cells, we found an overall negative correlation for HSC-associated genes. The correlation was significant for *MEIS1D* (*Online Supplementary Table S3*, *Online Supplementary Figure S1*). *Vice versa*, a positive correlation was detected between tran-

scripts downregulated in CD133+ cells and transcripts upregulated upon *MEIS1* overexpression, and this correlation was significant for *MEIS1B* (*Online Supplementary Figure S1*). The positively correlated genes included the erythroid markers *KLF1*, *HBB* and *RHAG* (*Online Supplementary Table S3*). Taken together, these findings further confirm that *MEIS1* expression induces a progenitor fate in HSC with a bias towards the megakaryocyte-erythroid lineage.

Discussion

While the critical role of Meis1 in maintaining quiescence and self-renewal capacity in HSC has been demonstrated earlier,[9,29-31] little is known about the role of this transcription factor in more committed progenitor cells. MEIS1 expression decreases when HSC differentiate to multipotent progenitors, but increases again during megakaryopoiesis. Here, we show that MEIS1 is important for the lineage commitment towards the MEP fate. Erythroid and megakaryocytic colony formation correlated with MEIS1 expression, both in total CD34+ cells and in common myeloid progenitors enriched from CD34+ cells. To understand the transcriptional changes inducing the observed bias towards a MEP fate, we performed gene expression profiling which indicated that *MEIS1* expression increased the expression of multiple megakaryocyte-erythroid-specific genes and downregulated genes controlling myeloid differentiation and stem cell expansion.

While we showed that, in adult human hematopoiesis, MEIS1 exerts its effects at the level of the CMP and GMP, other studies also indicated a role for Meis1 downstream of the CMP in the skewing of MEP towards the megakaryocytic lineage at the expense of erythroid commitment.[30] These differences can be explained in several ways. First, the study by Cai *et al.* was performed in a model system resembling primitive hematopoiesis whereas we investigated adult hematopoiesis. The transcription factor composition and transcription factor complexes that MEIS1 contributes to in these different cell types may therefore differ leading to divergent results. Second, in the study by Cai *et al.*, hematopoietic colonies derived from embryonic stem cells were cultured in semisolid media using a mix of cytokines (TPO, SCF, IL-3, IL-6, VEGF, Flt3 but lacking erythropoietin) which impairs the ability of embryonic stem cells to differentiate into the erythroid lineage and promotes differentia-

Table 1. ***MEIS1*** **expression maintains a progenitor cell fate thus enabling multilineage differentiation. Top 60 downregulated and upregulated transcripts after** ***MEIS1*** **overexpression in CD34+ cells. Fold changes from three independent experiments relative to empty vector controls are indicated.**

Upregulated genes			Downregulated genes		
Gene name	MEIS1B	MEIS1D	Gene name	MEIS1B	MEIS1D
HBD	2.31	1.52	*IGLL1*	-1.94	-1.52
TPSB2	2.27	1.67	*PROM1*	-1.69	-1.42
THBS1	2.25	1.46	*ATP9A*	-1.57	-1.37
TPSAB1	2.17	1.84	*CTSG*	-1.50	-1.36
TPM1	2.03	1.91	*LOC729760*	-1.49	-1.37
TPSAB1	1.96	2.11	*CD48*	-1.48	-1.37
TPM1	1.96	2.13	*LOC284422*	-1.47	-1.36
HBG1	1.94	1.64	*ICAM3*	-1.44	-1.40
APOC1	1.94	1.60	*LOC648343*	-1.43	-1.37
NEFM	1.84	1.68	*LOC442597*	-1.43	-1.61
LAT	1.80	1.59	*FAM26F*	-1.42	-1.64
SNORD46	1.77	1.57	*GLIPR1*	-1.41	-1.44
HBG2	1.75	1.50	*LOC645452*	-1.41	-1.43
EGR1	1.71	1.45	*MIR1299*	-1.41	-1.40
VWA5A	1.70	1.74	*LYRM1*	-1.40	-1.48
SPTA1	1.69	1.58	*NUDT10*	-1.40	-1.47
ABCC4	1.69	1.66	*C11ORF63*	-1.39	-1.67
FCER1A	1.67	1.57	*HNRNPU*	-1.39	-1.46
CNRIP1	1.67	1.65	*UGP2*	-1.39	-1.40
HBB	1.66	1.70	*MAGT1*	-1.39	-1.53
HBA2	1.66	1.45	*ATP8B4*	-1.39	-1.43
PRG2	1.65	1.48	*PTGR2*	-1.39	-1.38
FHL2	1.65	1.67	*LOC100128460*	-1.39	-1.40
GMPR	1.64	1.51	*GNB4*	-1.38	-1.41
IGFBP4	1.63	1.48	*TFAMP1*	-1.38	-1.40
PROS1	1.63	1.46	*RPL35A*	-1.38	-1.52
MTSS1	1.63	1.51	*SPTBN1*	-1.38	-1.37
LMNA	1.63	1.51	*LOC729004*	-1.38	-1.41
SYTL4	1.62	1.68	*MYO1B*	-1.38	-1.42
SLC40A1	1.61	1.48	*KLK7*	-1.38	-1.41
EMP3	1.61	1.58	*AK2*	-1.37	-1.39
TUBB2A	1.61	1.69	*RPLP0P2*	-1.37	-1.37
SLC40A1	1.60	1.47	*LOC146053*	-1.37	-1.39
GATA2	1.59	1.57	*ELANE*	-1.37	-1.44
CCND1	1.59	1.52	*KIAA0125*	-1.37	-1.38
ABCC4	1.59	1.88	*CD34*	-1.37	-1.58
LMO4	1.58	1.90	*PLAC8*	-1.37	-1.43
HBE1	1.58	1.46	*LOC731170*	-1.37	-1.36
SKIL	1.58	1.44	*CYLC2*	-1.37	-1.44
FBLN2	1.58	1.46	*FLJ41562*	-1.37	-1.56
LXN	1.57	1.45	*A1BG*	-1.37	-1.45
LAT	1.56	1.54	*FLJ32679*	-1.37	-1.38
PMP22	1.55	1.49	*HFE*	-1.37	-1.37
CD68	1.55	1.52	*RHBDL2*	-1.37	-1.39
SNORD48	1.54	1.60	*IL17RD*	-1.36	-1.96
SLC18A2	1.54	1.49	*LOC645307*	-1.36	-1.40
CTTNBP2	1.54	1.47	*GAGE1*	-1.36	-1.42
PEAR1	1.54	1.95	*ACBD7*	-1.36	-1.39
TMBIM1	1.54	1.60	*LOC441073*	-1.36	-1.38
PKNOX1	1.53	1.49	*FLJ32790*	-1.36	-1.42
FBLN2	1.53	1.61	*PLIN5*	-1.35	-1.43
LOC441454	1.53	1.78	*XBP1*	-1.35	-1.45
IL7	1.52	1.69	*RPS27L*	-1.35	-1.42
TMOD1	1.51	1.98	*STAG3L2*	-1.35	-1.37
BLVRB	1.51	1.59	*LOC100132804*	-1.35	-1.53
F2RL3	1.51	1.50	*P2RY8*	-1.35	-1.83
FTH1	1.51	2.03	*APBB2*	-1.35	-1.85
CCND3	1.50	1.46	*LOC389517*	-1.35	-1.36
CMIP	1.50	1.55	*NRIP1*	-1.35	-1.43

tion into the megakaryocytic lineage. This explains the impairment in erythroid progenitor cells that they observed as compared to the enhanced megakaryocyte differentiation. Third, only in CD41$^+$ cells did MEIS1 overexpression substantially decrease erythrocyte-specific genes and enhance megakaryocyte-specific genes. This indicates that MEIS1 only suppresses erythroid differentiation in already committed megakaryocyte progenitor cells.

On the other hand, even though the results of our study and that by Cai *et al.* differ, in both cases a clear effect of MEIS1 on cell proliferation was detected. The effect of MEIS1 on proliferation has also been reported earlier in the context of retina development[24] indicating a robust and conserved role for MEIS1 in proliferation that seems to be tissue-independent.

We further investigated the dynamics of the observed bias towards the MEP fate using expression profiling of *MEIS1*-overexpressing CD34$^+$ cells. It has been postulated that HSC can directly differentiate towards megakaryocytes.[32] Therefore, *MEIS1* could first reestablish a HSC fate from which the cells differentiate into the megakaryocyte-erythroid lineage. However, when CD34$^+$ cells were sorted into stem and progenitor cells, overexpression of *MEIS1* in the HSC subset did not result in a lineage bias towards a MEP fate. Furthermore, expression profiling showed downregulation of genes associated with a HSC fate, including *PROM1* (*CD133*),[28] *AK2*, and even *CD34*, indicating that the observed bias towards a MEP fate upon *MEIS1* overexpression is not caused by inducing a HSC fate first.

MEIS1 could also inhibit GMP differentiation, which by itself may cause a bias towards MEP. Indeed we showed that ectopic *MEIS1* blocked the differentiation into the granulocytic-monocytic lineage and decreased the expression of specific granulocytic genes such as *CD48* and elastase (*ELANE*) in CD34$^+$ cells. This hypothesis is supported by the study of Calvo *et al.*, who reported that *MEIS1* expression is able to inhibit granulocyte colony-stimulating factor-dependent granulocytic differentiation of immortalized progenitor cells, although this effect was only observed upon co-expression with *HOXA9*.[33] However, we also found genes whose expression was slightly increased upon *MEIS1* overexpression in GMP, such as the monocyte-specific gene *CD68*. Instead, the clear induction of erythroid-megakaryocyte-specific genes strongly suggests that *MEIS1* overexpression directly promotes a MEP fate.

Two *MEIS1* splice variants, *MEIS1B* and *MEIS1D*, are expressed in hematopoietic stem and progenitor cells and their expression decreases from the most immature stem cells to more committed progenitors.[13,17] Structurally, the two splice variants both contain the N-terminal PBX/HOX-interacting domain, a central DNA-binding homeodomain, and two C-terminal transactivation domains, but *MEIS1B* has an additional transactivation domain which regulates the transcriptional activity of *MEIS1*.[16,18] Upon trimerization with PBX1 and HOXA9, MEIS1 maintains the transcriptional activity of the complex by inhibiting nuclear export and promoting nuclear import.[34,35] Using separate overexpression of the splice variants, we showed that both induce the same phenotype, a skewing of CMP and GMP towards a MEP fate. Expression profiling further revealed that both splice variants control expression of many common transcripts, strongly indicating overlapping functions. However, in most experiments we observed a stronger phenotype upon overexpression of *MEIS1D*, suggesting more efficient regulation of gene expression by *MEIS1D*. Earlier studies also investigated the role of *Meis1* splice variants, using a model of primitive hematopoiesis. In this setting, it was found that the expression of Meis1b, which is equivalent to MEIS1D in our study, induced significant skewing of MEP towards the megakaryocytic lineage.[30] This further underlines the stronger transcriptional activity of *MEIS1D*.

Table 2. Comparison of gene expression between Haematlas lineage-specific genes and microarray on CD34$^+$ cells after *MEIS1* overexpression. Numbers of genes overlapping between microarray on CD34$^+$ cells and Haematlas lineage-specific genes.

Subset	Number of genes	
	Haematlas	MEIS1 array
CD4 specific	51	7
CD8 specific	7	0
CD14 specific	206	55
CD19 specific	260	38
CD56 specific	86	9
CD66b specific	906	210
Erythroblast specific	335	101
Megakaryocyte specific	289	109

While it has been shown that Meis1 depletion in murine HSC results in loss of quiescence and increased cell cycle entry,[9] it is less clear whether MEIS1 also affects the cell cycle of more committed progenitor cells. We found that silencing *MEIS1* resulted in almost no erythroid colonies, while granulocytic colony numbers were unaltered. This implies an important role for *MEIS1* in the expansion of erythroid progenitor cells. These results were confirmed in liquid cultures in which *MEIS1* also affected erythroid expansion although at later stages of erythropoiesis, MEIS1 expression declined. Comparative transcriptional profiling showed that *MEIS1* induces expression of *CCND1* and *CCND3* in CD34$^+$ cells which are linked to HSC and MEP expansion.[36,37] In addition, *CCND3* was also found to be essential for megakaryopoiesis, promoting progression through the G1-phase of the cell cycle.[38] These results strongly suggest that *MEIS1* reinforces MEP expansion and megakaryocytic differentiation via induction of *CCND1* and *CCND3*. Of note, Meis1 has also been found to regulate *ccnd1* expression during eye development in zebrafish indicating a strong, tissue-independent, regulation of *Ccnd1* by Meis1.

In conclusion, we show that in hematopoietic progenitor cells, MEIS1 is critical for the commitment towards the megakaryocytic and erythroid lineages by inducing expression of lineage-specific genes. Expression profiling of CD34$^+$ cells after MEIS1 overexpression revealed transcriptional changes in genes with currently unknown functions in lineage commitment or differentiation. Given the biphasic expression of MEIS1 in both hematopoietic stem and progenitor cells and adult megakaryocytes but not erythrocytes, further studies should focus on the effects of MEIS1 and its target genes on early and late megakaryocytic differentiation.

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