Homeodomain Transcription Factor Meis1 Is a Critical Regulator of Adult Bone Marrow Hematopoiesis

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Abstract

Hematopoietic stem cells in the bone marrow have the capacity to both self-renew and to generate all cells of the hematopoietic system. The balance of these two activities is controlled by hematopoietic stem cell-intrinsic regulatory mechanisms as well as extrinsic signals from the microenvironment. Here we demonstrate that Meis1, a TALE family homeodomain transcription factor involved in numerous embryonic developmental processes, is selectively expressed in hematopoietic stem/progenitor cells. Conditional Meis1 knockout in adult hematopoietic cells resulted in a significant reduction in the hematopoietic stem/progenitor cells. Suppression of hematopoiesis by Meis1 deletion appears to be caused by impaired self-renewal activity and reduced cellular quiescence of hematopoietic stem/progenitor cells in a cell autonomous manner, resulting in stem cell exhaustion and defective long-term hematopoiesis. Meis1 deficiency down-regulated a subset of Pbx1-dependent hematopoietic stem cell signature genes, suggesting a functional link between them in the maintenance of hematopoietic stem/progenitor cells. These results show the importance of Meis1 in adult hematopoiesis.

Citation: Ariki R, Morikawa S, Mabuchi Y, Suzuki S, Nakatake M, et al. (2014) Homeodomain Transcription Factor Meis1 Is a Critical Regulator of Adult Bone Marrow Hematopoiesis. PLoS ONE 9(2): e87646. doi:10.1371/journal.pone.0087646

Editor: Kevin D. Bunting, Emory University, United States of America

Received October 2, 2013; Accepted December 26, 2013; Published February 3, 2014

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Funding: This work has been supported by Grant-in-Aid for Scientific Research (21658105 and 25111513 to R. G.) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors declare no competing financial interests.

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Introduction

Hematopoiesis in adult animals is sustained by a small population of multipotent hematopoietic stem cells (HSCs), which maintain the capacity for both self-renew and differentiation, thereby generating all the cell types of the hematopoietic system. In normal mice and humans, HSCs are localized predominantly in a specialized microenvironment (niche) within the bone marrow (BM), where signals from cells in the surrounding niche maintain them in a state of slow cell cycling or quiescence [1–3]. The self-renewal of postnatal HSCs is closely coupled with this slow cell cycling or quiescence and is a critical requirement for long-term maintenance of the self-renewing HSC compartment.

HSC quiescence is controlled by both HSC-intrinsic mechanisms and extrinsic factors from the BM microenvironment [1]. Several transcription factors have been implicated in the regulation of HSC quiescence, including Gfi-1, Pbx1 and MEF/ELF4 [4–7]. With regard to HSC-extrinsic niche-derived factors, it has been reported that angiopoietin-1 and thrombopoietin regulate the quiescence of HSCs in the BM through receptors expressed on HSCs [8–10]. Furthermore, hypoxia inducible factor-1α (HIF-1α), a transcription factor that is transcribed and stabilized under low oxygen conditions such as in the BM niche for HSCs, has been shown to regulate HSC quiescence as well as metabolism [11,12]. Thus it is an important molecular link between extrinsic and intrinsic regulatory mechanisms modulating HSC quiescence.

The Meis1 gene encodes a TALE-family transcription factor that was first identified as a common retroviral integration site in BXH2 murine myeloid leukemia [13,14]. Meis1 functions as a DNA-binding cofactor of Hox proteins through interaction with Pbx, a member of another TALE homeodomain subfamily of transcription factors [15]. Meis1 by itself does not transform hematopoietic cells. However, it cooperates with Hoxa9 to significantly accelerate Hox-induced leukemogenesis [16]. Moreover, Meis1 as well as Hoxa9 have been shown to be the most critical downstream targets of Mixed Lineage Leukemia (MLL) fusion proteins [17], and their co-expression is sufficient to induce acute myeloid leukemia [14,16], recapitulating MLL-ENL-induced immortalization of myeloid progenitor cells [18]. In addition, Meis1 regulates the differentiation arrest, cycling activity and self-renewal of MLL leukemia cells, a critical rate-limiting determinant for establishing leukemia stem cell potential [19].

In contrast to the established role of Meis1 in leukemia development, its function in postnatal hematopoiesis, especially in HSCs as well as hematopoietic progenitor cells (HPCs), remains uncertain. Targeted Meis1 homozygous deletion in mice results in lethality by embryonic day 14.5 with hematopoietic and vascular defects [20,21]. In Meis1-deficient fetal liver, HSC compartments were severely affected and colony formation potential as well as the capacity to repopulate lethally irradiated recipient mice were...
profundely impaired, suggesting a critical role of Meis1 in HSC/HPC maintenance. Furthermore, Meis1 is required for transcriptional activation of Hlf in HSCs through binding to its conserved consensus sequence within the first intron of Hlf [11]. Thus, these observations support the hypothesis that Meis1 has a critical role in the regulation of HSC/HPC maintenance. However, a comprehensive analysis of Meis1 function has been hampered because of the embryonic lethality of the Meis1 mutation.

In the present study, we employed a genetic approach to conditionally inactivate Meis1 in the mouse hematopoietic system in vivo. Our current analysis reveals that Meis1 is a critical regulator of hematopoiesis in the adult BM.

Results

Impaired hematopoiesis in the absence of Meis1

As predicted from a gene expression database search [22], Meis1 was highly expressed in both CD34- and CD34+Lin-Sca-1-c-Kit+ (LSK) cells, whereas its expression became undetectable in most of the lineage-committed hematopoietic cells (Figure S1). Meis2 and Meis3 transcripts were undetectable in any of the hematopoietic lineage cells tested, therefore Meis1 is the sole Meis transcription factor family member expressed in hematopoietic cells under physiological conditions.

The early embryonic lethality resulting from germ-line deletion of the Meis1 gene precludes any study of postnatal hematopoiesis in the BM. Therefore, we generated mice harboring conditional alleles of Meis1 (Meis1fl/fl), in which Meis1 exon 8 encoding the homeodomain was flanked by loxP sites (Figure S2A and B). The Meis1fl/fl mice were born normally and appeared healthy. Given the expression pattern of Meis1, we chose to study the consequence of Meis1 ablation in the HSC/HPC by crossing the Meis1fl/fl conditional-knockout strain with the interferon-responsive Mx1-Cre transgenic line, which achieves highly efficient excision of loxP-flanked DNA in hematopoietic cells in vivo after induction with poly(I:C) [23]. As shown in Figure S2C, four intraperitoneal injections of poly(I:C) into Meis1Cre Meis1fl/fl mice was sufficient to induce complete deletion of Meis1 exon 8 in BM cells. Since both Meis1Cre Meis1fl/fl and Meis1fl/fl mice displayed similar phenotypes upon poly(I:C) treatment (data not shown), we used Meis1fl/fl mice as controls unless otherwise indicated.

We analyzed hematopoiesis in adult Meis1Cre Meis1fl/fl mice three weeks after poly(I:C) treatment compared to similarly treated Meis1fl/fl littermates. Three weeks after induced deletion of Meis1, the total number of BM cells was slightly but significantly reduced in Meis1Cre Meis1fl/fl mice (Figure 1A and B). At the HSC/HPC level, LSK cells were almost undetectable in Meis1Cre Meis1fl/fl mice (Figure 1A and B). The relative proportion and the total number of Lin-IL-7Ralpha-Sca-1int-c-Kit+ (CLP) cells was significantly lower in Meis1Cre Meis1fl/fl mice than in control Meis1fl/fl mice (Figure 1A and B). In addition, the Lin-Sca-1-c-Kit/FcγRIIa/IIIint CD34+CD41+ (CMP) population was nearly absent, and the cell populations at the subsequent developmental stages, Lin-Sca-1-c-Kit/FcγRIIa/IIIint CD34+CD41+ (GM) as well as Lin-Sca-1-c-Kit/FcγRIIa/IIIint CD34+CD41+ (MEP), were also significantly reduced in Meis1Cre Meis1fl/fl mice compared to control Meis1fl/fl mice (Figure 1A and B).

In contrast to the profound decrease in lineage-negative hematopoietic precursors, the frequencies and the total numbers of granulocytes (Gr-1+CD11b+) was unaffected in Meis1Cre Meis1fl/fl mice and the number of monocytes (Gr-1+CD11b+) was only slightly reduced (Figure 1C and D), in spite of the efficient deletion of the floxed Meis1 alleles in most of these cells (Figure S3). The differentiation profile of erythroid lineage cells from proerythroblast (I; Ter119low CD71high), basophilic erythroblast (II; Ter119low CD71high), to late erythroblasts (III; Ter119high CD71low, and IV; Ter119high CD71high) was also intact in Meis1Cre Meis1fl/fl mice (Figure 1C and D). Furthermore, consistent with the essential role of Meis1 in fetal megakaryopoiesis [20,21], the proportion and number of Lin- c-Kit+CD41+ megakaryocyte precursors in the BM, which contain CFU-Meg [24], was also significantly reduced in Meis1Cre Meis1fl/fl mice; however, at this time point after Meis1 deletion, the number of c-Kit+CD41+ mature megakaryocytes was unaffected in Meis1Cre Meis1fl/fl mice (Figure S4), as observed in other lineage-committed cells. The proportions and the total numbers of early B-lineage cells containing pro- and pre-B cell populations (B220low IgM-) as well as immature B cells (B220low IgM+) in the BM were also significantly reduced in Meis1Cre Meis1fl/fl mice (Figure 1C and D), which is consistent with the relatively low expression of Meis1 in bone marrow B220+ B-lineage cells (Figure S1). T cell numbers in the thymus were also significantly reduced in Meis1Cre Meis1fl/fl mice compared to those in the control mice (Figure S5). These results demonstrated that acute Meis1 loss results in severe defects in early hematopoiesis at the progenitor levels rather than in late lineage-committed cells.

Defects in the HSC compartment in the absence of Meis1

The LSK population is heterogeneous and contains both HSCs and HPCs with multi-lineage potential but limited self-renewal capacity [25,26]. Therefore, we examined the LSK compartment in detail by staining for CD34 or Flt3 expression. The total number of CD34- LSK cells was lower in Meis1Cre Meis1fl/fl mice than in control Meis1fl/fl mice (Figure 2A and B), and the defect was more profound in CD34+ LSK cells as well as in Flt3- and Flt3+ LSK cells (Figure 2A and B). CD34- LSK cells as well as Flt3+ LSK cells were about 20-fold and 100-fold lower in Meis1Cre Meis1fl/fl mice than in control Meis1Cre Meis1fl/fl mice, respectively (Figure 2A and B). Furthermore, “side population” (SP) cells, which represent quiescent HSCs [27], but not non-SP cells, in the LSK population were almost completely missing from Meis1Cre Meis1fl/fl mice upon induction of Meis1 deletion (Figure 2C). These results suggest that the stages among LSK cells that require Meis1 regulate self-renewal of HSC in a cell autonomous manner.

Although the above data suggested that Meis1 deficiency caused a loss of HSC/HPCs, Meis1-Cre-mediated induction of Meis1 loss is not strictly limited to hematopoietic cells [23]. Thus, to determine whether the Meis1-deficient phenotype is HSC autonomous or dependent on the lack of Meis1 in the HSC niche, we induced the loss of Meis1 in pre-established BM chimeric mice. Irradiated recipient mice (CD45.1+) were transplanted with a 1:1 mixture of donor CD34- LSK cells from uninduced Meis1Cre Meis1fl/fl mice or control Meis1fl/fl mice (CD45.2+) and competitor CD34- LSK cells from wild-type mice (CD45.1/CD45.2). Three months later, chimeric mice were injected with poly(I:C) to induce Meis1 loss, and the fraction of donor-derived peripheral blood leukocytes (PBL) was subsequently monitored (Figure 3A). In contrast to untreated recipient mice, the poly(I:C)-injected recipients manifested a sharp decline of Meis1Cre Meis1fl/fl cell-derived PBL, as compared to control Meis1fl/fl cell-derived PBL (Figure 3B). Three months after induction of Meis1 deletion, a significant reduction of Meis1-deficient donor cells of all hematopoietic cell types, including B cells, T cells, monocytes and granulocytes in the spleen was confirmed (Figure 3C). Furthermore, Meis1-deficient donor cells were undetectable from one to three months after the
Figure 1. Loss of Meis1 leads to a depletion of hematopoietic progenitor cells from the bone marrow. (A) Representative flow cytometric profiles of hematopoietic progenitor cell populations from Mx1-Cre\textsuperscript{+} Meis1\textsuperscript{fl/fl} and control Meis1\textsuperscript{fl/fl} mice three weeks after poly(I:C) treatment. Gates used to identify progenitor populations are outlined, and rightward arrows within the plots indicate their relationship to subsequent plots showing the progenitor populations. Numbers adjacent to outlined areas indicate percentage of gated cells in total BM mononuclear cells. (B) Absolute numbers of the indicated cell populations per two femurs in poly(I:C)-treated Mx1-Cre\textsuperscript{+} Meis1\textsuperscript{fl/fl} (solid bars) and control Meis1\textsuperscript{fl/fl} (open bars) mice (mean and SD; n = 4). (C) Representative flow cytometric profiles of lineage-committed cell populations. (D) Absolute numbers of the indicated cell populations per two femurs in poly(I:C)-treated Mx1-Cre\textsuperscript{+} Meis1\textsuperscript{fl/fl} (solid bars) and control Meis1\textsuperscript{fl/fl} (open bars) mice (mean and SD; n = 4). Pro- and pre-B cells (B220\textsuperscript{low} IgM\textsuperscript{+}), immature B cells (B220\textsuperscript{low} IgM\textsuperscript{+}), mature B cells (B220\textsuperscript{high} IgM\textsuperscript{+}), granulocytes (Gr-1\textsuperscript{+}CD11b\textsuperscript{+}), monocytes (Gr-1\textsuperscript{+} CD11b\textsuperscript{+}), proerythroblasts (I; Ter119\textsuperscript{low} CD71\textsuperscript{high}), basophilic erythroblast (II; Ter119\textsuperscript{high} CD71\textsuperscript{high}) and late erythroblasts (III; Ter119\textsuperscript{high} CD71\textsuperscript{high} and IV; Ter119\textsuperscript{high} CD71\textsuperscript{low}). *p<0.05 and **p<0.01.

doi:10.1371/journal.pone.0087646.g001
Figure 2. Loss of Meis1 causes profound defects in the HSC compartment. (A) Representative flow cytometric profiles of LSK cells in BM depleted of lineage-positive cells from Mx1-Cre<sup>+</sup> Meis1<sup>fl/fl</sup> and control Meis1<sup>fl/fl</sup> mice three weeks after poly(I:C) treatment. Gates used to identify LSK cell populations are outlined, and downward arrows indicate their relationship to subsequent histograms showing the expression of CD34 or Flt3 in these cell populations. Numbers in the histograms indicate percent events in each gate. (B) Absolute numbers of the indicated LSK cell populations per two femurs from poly(I:C)–treated Mx1-Cre<sup>+</sup> Meis1<sup>fl/fl</sup> (solid bars) and control Meis1<sup>fl/fl</sup> (open bars) mice (mean and SD; n = 4). *p<0.05 and **p<0.01. (C) Representative flow cytometric profiles of side population (SP) cells within the LSK population from Mx1-Cre<sup>+</sup> Meis1<sup>fl/fl</sup> and control mice one week after poly(I:C) treatment. Bar graphs shown on the right represent the percentage of SP cells in the LSK cell population from poly(I:C)–treated Mx1-Cre<sup>+</sup> Meis1<sup>fl/fl</sup> (solid bars) and control Meis1<sup>fl/fl</sup> (open bars) mice (mean and SD; n = 3). *p<0.005.

doi:10.1371/journal.pone.0087646.g002

Meis1 Maintains the Adult Hematopoiesis
Figure 3. Meis1 regulates self-renewal of HSCs in a cell autonomous manner. (A) Experimental strategy for analyzing the function of Meis1 in HSCs. Mice with chimeric BM were generated by transplanting CD34^+ LSK cells (50 cells/mouse) from Mx1-Cre^+ Meis1^fl/fl or control Meis1^fl/fl mice (CD45.2) and an equal number of CD34^+ LSK cells from wild-type mice (CD45.1/CD45.2) with CD45.1 BM support cells in lethally irradiated CD45.1 recipient mice. A subset of mice was treated with poly(I:C) three months after transplantation. (B) Mean percentages of CD45.2^+ cells ± SD in the peripheral blood derived from Meis1^fl/fl (n = 6; open circles) and Mx1-Cre^+ Meis1^fl/fl (n = 6; closed circles) CD34^+ LSK cells after poly(I:C) treatment. Initial
Figure 3D. These data indicate that Meis1 regulates the long-term maintenance of HSC/HPCs in a cell autonomous manner.

We next examined the impact of Meis1 loss on HSC/HPC proliferation and differentiation using in vitro colony forming assays. As shown in Figure 3E, Meis1−/− CD34+ LSK cells generated ten-fold fewer colonies compared to control Meis1fl/fl CD34+ LSK cells. Furthermore, colonies derived from Meis1−/− CD34+ LSK cells were significantly smaller in size than those from the control cells, indicating that Meis1-deficient cells had lost replication potential (Figure 3F). Despite the significant decrease in the number and size of colonies derived from Meis1-deficient cells, they still retained the potential to give rise to CFU-GM, CFU-M, CFU-G and BFU-E, but failed to form CFU-GEMM colony formation. The data are the means of three-independent experiments. (F) Representative photographs of 14 day cultures were assessed on day 14 for granulocyte (CFU-G), monocytes (CFU-M), granulocyte-monocyte (CFU-GM), erythroid (BFU-E) cell formation. The data are the means of three-independent experiments. doi:10.1371/journal.pone.0087646.g003

**Discussion**

Despite the overwhelming evidence that Meis1 is involved in leukemogenesis, its normal physiological functions remain unclear. By using an inducible conditional knockout approach in adult mice, we have demonstrated here that Meis1 deletion results in a loss of HSC/HPCs, subsequently causing multi-lineage BM failure. Specific deletion of Meis1 within the hematopoietic system demonstrates a cell-autonomous requirement for Meis1 in maintaining the adult HSC/HPCs. In the HSC compartment, loss of Meis1 enhanced cell cycle entry, with almost complete loss of the most quiescent SP cells, although cell survival was not affected. Together, our data provide evidence that Meis1 functions in maintaining long-term hematopoiesis via regulating the cell cycle status of HSC/HPCs.

Quiescence of stem cells has been postulated to prevent their exhaustion and is tightly linked to maintenance of the long-term self-renewal capacity of tissue stem cells, including HSCs [29]. Several transcriptional regulators have been shown to play key roles in this process. In the absence of Meis1, cells in the HSC compartment appear to exit from their quiescent state, as evidenced by the loss of the SP fraction from LSK cells, and undergo cell-cycle entry without an increase in this compartment, suggesting that Meis1 regulates self-renewal capacity of HSCs but not their simple expansion or differentiation. This notion was further supported by our in vitro colony formation assays using CD34+ LSK cells, in which Meis1 deficiency significantly reduced the numbers and size of colonies but did not affect their potential to differentiate into various types of colonies, with an exceptional lack of mixed type colonies. Therefore, we postulate that Meis1 functions in replication of HSC/HPCs rather than in their differentiation. In this regard, a similar cell context-dependent role in cell cycle control was observed in MLL1-deficiency, in which the loss of MLL1, a potential upstream regulator of Meis1 in the hematopoietic cell compartment, induced cell cycle progression in the HSC compartment whereas it reduced the proliferation of progenitor populations [30]. Thus, it is possible that Meis1 is a core molecule downstream of MLL1 that regulates the self-renewal capacity of HSCs via regulating their cell cycle status.

Another important protein that is associated with Meis1 in the HSC compartment and is possibly involved in cell cycle regulation is Pbx1, a directly interacting Meis1 partner in DNA binding [13]. Pbx1 is the most highly expressed Pbx family member in HSCs [11]. Its expression was not significantly affected upon Meis1 deletion in LSK cells. However, the expression of Bcl2 in Meis1-deficient LSK cells was reduced by about eight-fold, compared to that in controls (Table S1), an observation that is consistent with a previous observation that Bcl2 is a direct transcriptional target of Meis1 [29]. We also quantified the expression of cell cycle-related genes. Although no statistical differences in the expression of Cdkn1b (p27), Cdkn2b (p13), or Cdk6 were detected (data not shown), Cnd1 (cyclin D1) expression was significantly increased in Meis1-deficient LSK cells, when compared to that in sham-treated control LSK cells (Figure 3A). Taken together, these findings indicate that Meis1 regulates genes involved in the self-renewal and cell cycle of HSC/HPCs.

Meis1 regulates the cell cycle status of HSC/HPCs

To understand the mechanism of HSC/HPC exhaustion in the absence of Meis1, we assessed apoptosis of HSCs using Annexin V staining of LSK cells at an early time point (one week) after deletion of Meis1, when no decrease in cell numbers or increase in apoptosis of any hematopoietic cell compartments, including LSK cells and Lin− Sca1− c-Kit− (LK) cells, was detectable (data not shown). As shown in Figure 4A, we found no difference in the proportion of Annexin V+ LSK cells between Meis1−/− and control Meis1fl/fl mice. We next examined cell cycle status of HSC/HPCs because it is important for maintenance of their long-term self-renewal capacity [29]. Cells that had entered S phase, as detected by BrdU incorporation, were markedly increased in Meis1−/− LSK cells compared with control Meis1fl/fl LSK cells, and this was accompanied by a significant reduction in the fraction of Meis1−/− LSK cells in G0/G1 (Figure 4B). Taken together, these data suggest that Meis1 regulates the cell cycle of HSC/HPCs, and its absence might lead to the exhaustion of HSC/HPCs.

Meis1 regulates expression of genes involved in HSC/HPC maintenance

Finally, we examined the impact of the loss of Meis1 on gene expression in LSK cells. An initial screening by global gene profiling of LSK cells from control and Meis1−/− mice one week following poly(LC) treatment revealed a profound reduction in the expression of several genes in Meis1-deficient LSK cells, including Egr2, Trnb2, Hmga2, Hif1α, Mitf3, Smad7, Sfnet2, Skil, and Ms22 (Table S1). These genes are also affected by Pbx1-deficiency in HSCs [5]. The change in expression of several genes was verified by quantitative PCR (Figure 5A). Although Hif1α was reported to be a direct transcriptional target of Meis1 in HSCs [11], its expression was not significantly affected upon Meis1 deletion in LSK cells. However, the expression of Bcl2 in Meis1-deficient LSK cells was reduced by about eight-fold, compared to
Figure 4. Meis1 regulates cell cycle of the HSC compartment. (A) Representative flow cytometric profiles showing Annexin V and 7-AAD staining of LSK cells from Meis1fl/fl and control mice one week after poly(I:C) treatment. Bar graphs on the right represent the percentages of apoptotic LSK cells (annexin V7-AAD+)) cells from poly(I:C)-treated Meis1fl/fl (solid bars) and control Meis1fl/fl (open bars) mice (mean and SD; n = 3). (B) Representative flow cytometric profiles showing BrdU incorporation and 7-AAD staining of LSK cells from poly(I:C)–treated one week after poly(I:C) treatment. Bar graphs shown on the right represent the percentages of cells in G0/G1-, S- and G2/M-phase of the cell cycle in apoptotic LSK cells (anexin V7-AAD+).
Meis1-deficient mice [40,41]. Although the HSC phenotypes they reported are almost identical with those described in the current study, the role of Meis1 in Hif1a expression was somewhat different among these three reports. The discrepancy regarding Meis1-mediated regulation of Hif1a expression in HSCs between our findings in the present study and those of both Unnisa et al. and Kocabas et al. could be due to differences in the cell type (LSK versus CD34− LSK or Lin− BM cells) or methods used for Meis1 deletion, interferon-mediated Mx1-Cre versus tamoxifen-mediated CreER, which may affect the relative contribution of Meis1 in regulating Hif1a expression. Thus, it is likely that Meis1-mediated Hif1a expression is strictly cell context- and/or differentiation stage-dependent. However, we cannot exclude a potential contribution of Hif1a in HSCs as one of the downstream targets of Meis1.

In conclusion, we demonstrate that Meis1 is required for the maintenance of adult hematopoiesis in the BM. Meis1 has also been reported to maintain the undifferentiated state and expansion of retinal progenitor cells [42,43], and the loss of Meis1 causes premature differentiation of these cells. Thus, further investigation of the molecular mechanisms underlying the functions of Meis1 in HSCs, such as its potential functional cooperation with Pbx1 and/or Hox, as well as its involvement in asymmetric/symmetric cell division of HSCs, should facilitate our understanding of transcriptional networks regulating the maintenance of stem cells as well as their neoplastic counterparts.

Figure 5. Meis1 controls expression of genes involved in HSC cell cycle and maintenance. (A) Alterations of gene expression in LSK cells induced by Meis1 loss were analyzed by quantitative RT-PCR. Histograms show the indicated transcripts in sorted LSK cells from Mx1-Cre+ Meis1fl/fl mice (open bars) and control Meis1fl/fl mice (solid bars) one week post poly(I:C) treatment. Data were normalized to Gapdh expression and the level of each transcript in LSK cells from control mice was arbitrarily set to 1. Data are the means and standard deviations of three independent experiments. *p<0.05 and **p<0.01. (B) Models illustrating potential mechanisms of Meis1 function in the maintenance of HSC.

doi:10.1371/journal.pone.0087646.g005
Meis1fl/fl Meis1fl/fl a FACSCalibur or a FACSCanto™II flow cytometers (BD Bioscience) was used for cell sorting.

**Materials and Methods**

**Ethics statement**

All animal experiments were carried out under the ethical guidance of Tokyo University of Science, and protocols were reviewed and approved by the Tokyo University of Science Animal Care and Use Committee.

**Mice and gene targeting**

The Meis1 targeting vector was assembled in a pKSTKloxP-Neo plasmid containing appropriate loxP sites, a loxP-flanked PGK promoter-driven neo gene, and the HSV thymidine kinase gene. The homologous regions of the final vector consisted of a 0.9 kb genomic fragment immediately upstream of the loxP-flanked 0.5 kb fragment containing exon 8 of the Meis1 gene and a 6.5 kb DNA fragment immediately downstream of the gene. To establish mice carrying the Meis1 floxed allele, the linearized targeting vector was electroporated into E14 ES cells, and drug-resistant colonies were screened for homologous recombination. Targeted clones were injected into C57BL/6 blastocysts and the resultant chimeric mice were bred to produce progeny capable of germ line transmission of the mutated allele. To remove the loxP-flanked neomycin-resistant gene cassette, mice harboring a targeted Meis1 allele with the neomycin-resistant gene (Meis1puro/+ ) were crossed with Elmo-Cre transgenic mice [44] and the resultant Meis1puro/+ Elmo-Cre (mosaic) mice were crossed with C57BL/6 mice to establish Meis1puro/+ mice and also Meis1fl/fl mice. Meis1fl/+ mice were backcrossed at least eight times onto the C57BL/6 background and then were crossed with Mx1-Cre [23]. Meis1-Cre Meis1fl/+ and Meis1fl/fl mice (controls) at the age of four to eight weeks were injected i.p. with 1.5 μg/weight (g) of poly(I:C) (GE Healthcare Bioscience) four times at one-day intervals to activate the interferon gene.

**Flow cytometry and cell sorting**

Single cell suspensions from the indicated organs were stained with a combination of FITC-, PE-, -APC, -APC-Cy7 and biotin-conjugated antibodies, followed by streptavidin-PerCP-Cy5.5 or streptavidin-PerCP-Cy5.5 (eBioscience, San Diego, CA). Conjugated and unconjugated antibodies specific for the following antigens were purchased from BD Biosciences (San Jose, CA) and eBioscience: TCRβ, CD3e, CD4, CD8, CD11b, Gr-1, TER119, CD253, FcγRII/III (2.4G2), Sca-1, c-Kit, MHC class II, CD44, and CD45.1, CD45.2, and CD45.3 (BD Bioscience). Sorted CD34+ LSK cells (100 cells) from Meis1-Cre+ Meis1fl/+ or Meis1fl/fl mice pretreated with poly(I:C) were plated in methylcellulose medium (Stem Cell Technologies) supplemented with mixtures of cytokines. The culture dishes were incubated at 37°C in a 5% CO2 humidified atmosphere, and colony numbers were counted at day 14. Colonies (>1 mm in diameter) were recovered and subjected to May-Grünewald Giemsa staining for morphological examination.

**Cell cycle and apoptosis analyses**

For bromodeoxyuridine (BrdU) incorporation analysis, BrdU (1 mg per mouse: BD Pharmingen) was injected intraperitoneally. At one h post-injection, LSK cells were collected from the BM, fixed, and stained with 7-AAD and anti-BrdU antibody using a FITC-BrdU Flow kit (BD Pharmingen), according to the manufacturer’s protocol. To assess apoptosis, cells stained for stem cell markers were further incubated with Annexin V and propidium iodide (PI).

**Microarray analysis**

RNA was isolated using the Qiagen RNeasy micro kit (Qiagen) from LSK cells from poly(I:C)-treated Meis1-Cre Meis1fl/+ and sham-treated Meis1fl/fl mice (pools of 6 mice; 2 pools per genotype). Ten ng of total RNA was amplified using the WT-Ovation™ Pico RNA Amplification system (NuGEN Technologies, Inc.) and labeled using the Genomic Enzymatic Labeling Kit (Agilent Technologies). Labeled probes were hybridized on 4 × 44 K Whole Mouse Genome Oligo Microarrays (Agilent) and scanned with an Agilent Microarray Scanner. Microarray signals and background information were retrieved using Feature Extraction Software (v.9.5.3.1). All data analyses were performed using the GeneSpring software GX11.0.2 (Agilent). Genes with a raw P-value<0.01 and a fold-change greater than 2-fold were defined as differentially expressed. Array data are available at Gene Expression Omnibus (GEO accession number: GSE38336).

**RT-PCR analysis**

Using the Qiagen RNeasy micro kit (Qiagen), total RNA was isolated from LSK cells from poly(I:C)-treated Meis1-Cre Meis1fl/+ and sham-treated Meis1fl/fl mice (pools of six mice; two pools per genotype). Total RNAs were reverse transcribed using a SuperScript VILO cDNA Synthesis System (Invitrogen, Carlsbad, CA). Real-time RT-PCR was performed with Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) and ABI 7500 Fast thermocycler (Applied Biosystems), according to the manufacturer’s protocol. Amplification of β-actin was used to normalize for sample RNA content. Specificity of products was confirmed by melting curve analysis, assessing band size in 2% agarose gels, and DNA sequencing. The primer sequences used for RT-PCR and qPCR are listed in Table S2.

**Statistical analysis**

Statistical significance was calculated using an unpaired two-tailed Student’s t-test. Data were considered statistically significant when p values were less than 0.05.
Supporting Information

Figure S1 Preferential expression of Meis1 in HSCs. Expression of Meis1, Meis2, Meis3, and GAPDH genes was examined by semiquantitative RT-PCR analysis. cDNAs were prepared from CD34+ LSK cells, CD34+ LSK cells, lineage marker- cells, Gr-1+ neutrophils, Mac-1+ macrophages, TER119+ erythroblasts, and B220+ B-lineage cells from BM; from B220+ B cells and CD3+ T cells from the spleen; and, from CD4+CD8− (DN), CD4+CD8+ (DP), CD4+CD8− (CD4 SP), and CD4+CD8+ (CD8 SP) T-lineage cells in the thymus of adult wild-type mice.

Figure S2 Generation of conditional and deleted Meis1 alleles. (A) A diagram depicting exon 8 of the Meis1 locus and the targeting strategy used to generate two targeted versions of the Meis1 allele (floxed and deleted alleles). LoxP sites (arrowheads) were inserted into intronic sites flanking exon 8 of the Meis1 gene. Correct targeting was verified by Southern blot analysis of HindIII-digested DNA with the indicated probe (filled rectangle). The lengths of the respective HindIII fragments are shown in kb. PCR primers for verifying the Cre-mediated deletion of the loxp-flanked fragment are indicated by arrows. Neo, neomycin-resistant gene; tk, thymidine kinase gene; H, Hind III. (B) Southern blot analysis of germline transmission of the mutated Meis1 alleles. Tail DNA from the indicated mice was digested with Hind III and hybridized with the probe indicated in (A). (C) Confirmation of Meis1 deletion in Mxl-Cre+ mice. DNAs from sorted LSK cells from Mxl-Cre+ Meis1fl/fl mice that were either treated (+) or untreated (−) with poly(I:C) were subjected to PCR analysis using primer pairs shown in (A). DNAs from Meis1fl/A mice were used as controls.

Figure S3 PCR genotyping of hematopoietic cells from Mxl-Cre+ Meis1fl/fl and control Meis1fl/fl mice three weeks after poly(I:C) treatment. Efficient excision of floxed Meis1 alleles was observed in sorted Gr-1+ CD11b+ mature granulocytes.

Figure S4 Loss of Meis1 abrogates megakaryocyte lineage differentiation in the bone marrow. Representative flow cytometric profiles of megakaryocytic-lineage cell populations from Mxl-Cre+ Meis1fl/fl and control Meis1fl/fl mice three weeks after poly(I:C) treatment. Gates used to identify megakaryocytic-lineage cell populations (Lin−) are outlined, and rightward arrows indicate their relationship to subsequent plots showing the megakaryocyte precursors (Kit+ CD41+) and mature megakaryocytes (cKit+ CD41+). Numbers within the analysis gates indicate percentage of gated cells in total BM mononuclear cells. Bar graphs on the right represent absolute numbers of the indicated cell populations per two femurs in poly(I:C)-treated Mxl-Cre+ Meis1fl/fl (solid bars) and control Meis1fl/fl (open bars) mice (mean and SD; n = 4). **p<0.01.

Table S1 The list of genes differentially expressed between Meis1-deficient and –sufficient LSK cells.

Table S2 The list of primers for qPCR.

Acknowledgments

We would like to thank Dr. K. Rajewsky for providing mice and Dr. Peter D. Burrows for critical reading of the manuscript.

Author Contributions

Conceived and designed the experiments: Y. Mattsuzaki TN RG. Performed the experiments: RA SM MN KY SH. Contributed reagents/materials/analysis tools: HN. Wrote the paper: TN RG.
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PLOS ONE | www.plosone.org 11 February 2014 | Volume 9 | Issue 2 | e87646