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研究課題名（和文）Development of novel therapeutic strategy against myeloid leukemia by inducing leukemia stem cell differentiation.

研究課題名（英文）Development of novel therapeutic strategy against myeloid leukemia by inducing leukemia stem cell differentiation

研究代表者

于　磊（YU, Lei）

東北大学・医学系研究科・産学官連携研究員

研究者番号：００７６０６５４

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研究成果の概要（和文）：本研究では、造血幹細胞・前駆細胞（HSPCs）から赤血球分化が開始する機構の一端を明らかにした。Gata1遺伝子を中心付近に含む全長196 kbの大腸菌人工染色体（BAC）クローンから、Gata1遺伝子HSPC特異的サイレンサーを欠失させたクローンを用いてトランスジェニックマウスを樹立することによって、HSPCにGATA1またはCreERT2酵素を誘導的に高発現させるシステムを構築した。これを用いて解析を行ったところ、Gata1遺伝子エンハンサーが脱メチル化されることによって、赤血球分化のマスター転写因子であるGATA1の発現が活性化することが赤血球分化開始の鍵となっていることが示された。

研究成果の概要（英文）：I identified the crucial mechanism of erythropoiesis initiation from hematopoietic stem and progenitor cells (HSPCs). By generating several lines of Gata1 bacterial artificial chromosome transgenic mice in which the HSPC specific silencer was deleted, I could be able to induce high level of Gata1 or CreERT2 expression in HSPCs. By using these mouse models as well as Dnmt1 conditional knockout allele, I found the demethylation of enhancers (which was methylated and repressed by the HSPC specific silencer) of erythroid master transcription factor GATA1, and therefore the gene activation, is the key of the initiation of red blood cell differentiation.

研究分野：　医化学

キーワード：　Gata1 gene regulation　Erythropoiesis　Apoptosis

１．研究開始当初の背景

Myeloid leukemia is a hematological malignancy, which is characterized with aberrant expansion of leukemic blast cells committed into myeloid lineage. Normal hematopoiesis is established by hierarchical differentiation of Hematopoietic Stem Cells (HSCs) to maintain homeostasis of hematopoietic system. HSCs are able to self-renew as well as give rise to all lineages of progenitors, which subsequently lose their self-renewing capacity and multi-potency. Because HSCs self-renew all life long, spontaneous genetic mutations tend to accumulate in the HSCs, which could potentially induce hematological malignancy. The mutated HSCs, which could give rise to all the leukemic blast cells, are designated as leukemic stem cells (LSCs). LSCs are rare population that can be resistant against chemotherapeutic agent and provoke disease relapse by reconstituting all the leukemia blasts.

Currently, the leukemia differentiation therapy aims to induce the differentiation of immature leukemia blast cells, which would yield leukemia remission. However, leukemia differentiation therapy is effective only in acute promyelocytic leukemia patient (APL, one subtype of acute myeloid leukemia). While the differentiation therapy of APL exerts efficacy through differentiation of immature granulocyte, no LSC-targeted differentiation therapy has been established, so far.

２．研究の目的

In this study, we initially try to develop a novel treatment for myeloid leukemia by enforcing differentiation of LSC through induction of GATA1. We hypothesized that enforced expression of erythroid and megakaryocytic master transcription factor GATA1 in LSCs would lead LSCs into differentiated erythroid/megakaryocytic lineages, which are sensitive to current leukemia chemotherapy.

３．研究の方法

Gata1 gene expression during erythropoiesis has been reported to require promoter-proximal CACCC motifs, a palindromic double-GATA motif (dbG) at approximately 650 bp upstream of the transcription start site (TSS) and a GATA motif in the *Gata1* gene hematopoietic enhancer (G1HE) at 3.7 kb upstream of the TSS. Our lab previously identified the Gata1 gene HSC-specific silencer element between G1HE and dbG (the Gata1 methylation determining region; G1MDR) that recruits Dnmt1 and provokes the methylation of the *Gata1* gene enhancer.

It has been shown that HSCs and LSCs share many similarities, for example in cell surface markers and gene expression profiles. In this regard, we assume that by generating transgenic mouse lines harboring a *Gata1* bacterial artificial chromosome (BAC), in which G1MDR was deleted, we could induce GATA1 gene expression in HSC as well as in LSC.

Three transgenic mouse lines that carry modified *Gata1* BAC DNAs in which the G1MDR was deleted to express either GATA1, GATA1-estrogen receptor variant (G1ER^{T2}) or Cre-estrogen receptor variant (CreER^{T2}) in stem cell fraction were generated. Mouse lines harboring these constructs individually are referred to as MG-G1, MG-G1ER^{T2}, and MG-CreER^{T2} respectively (Figure 1).

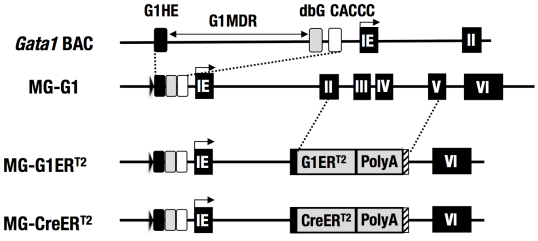

FIG 1. Generation of MG-G1, MG-G1ER^{T2} and MG-CreER^{T2} BAC transgenic mouse lines.

４．研究成果

4-1. High level of GATA1 induces erythroid-skewed hematopoiesis.

Flow cytometry of the fetal liver cells demonstrated that the percentages of LSK cells, CMPs and granulocyte-monocyte progenitors (GMPs) were significantly decreased, while the percentages of MEPs and ProEBs were slightly increased. The percentages of Mac1/Gr1+

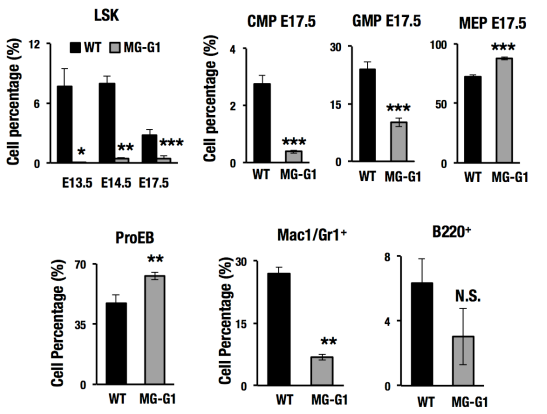

FIG 2. High level of GATA1 enforces embryonic hematopoiesis to erythroid lineage.

myeloid and B220+ B-lymphocytic cells were also lower. These results demonstrate that derepression of the G1MDR-mediated repression of Gata1 gene in HSPCs directed their differentiation toward erythroid lineages at the expense of the myeloid and lymphoid differentiation (Figure 2).

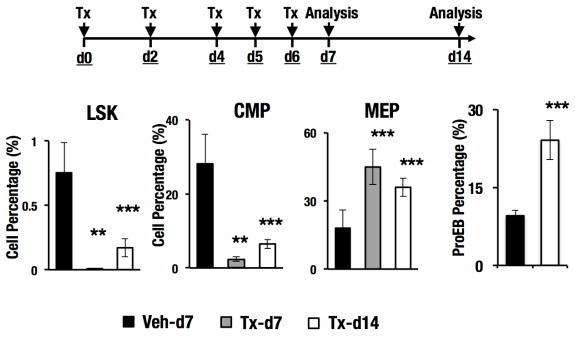

FIG 3. High level of GATA1 enforces bone marrow hematopoiesis to erythroid lineage.

To confirm the embryonic fetal liver data, we next generated a new mouse line harboring a tamoxifen-inducible GATA1-ERT2 fusion protein expressed under the regulation of the Gata1 MG-BAC (MG-G1ERT2, Figure 1). Consistent with MG-G1 fetal liver data, we found the erythroid-skewed differentiation in adult bone marrow of MG-G1ERT2 mice after tamoxifen induction (Figure 3).

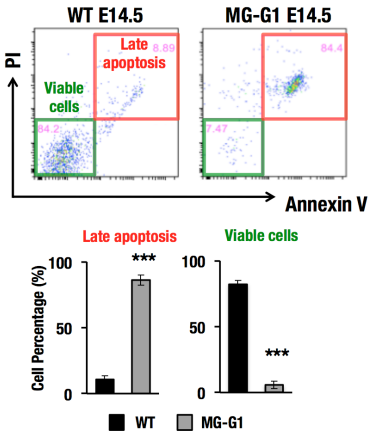

FIG 4. High level of GATA1 induce HSC apoptosis.

4-2. GATA1 induces HSC apoptotic cell death.

As induction of GATA1 expression is associated with depletion of HSC and progenitor, we next approached the issue of whether the elevated GATA1 expression in HSPCs actually participates in the program of apoptotic cell death. For this purpose, we examined the cell viability status of the LSK population in the livers of E14.5 MG-G1 embryos by Annexin-V/propidium iodide staining (Figure 4). These results clearly demonstrate thataberrant GATA1 activity induces HSPCs to undergo apoptosis in the MG-G1 embryos.

4-3. Dnmt1 conditional knockout recapitulates the phenotype of GATA1 over-expression.

To further clarify Gata1 gene regulation in HSPCs, we generated two lines of MG-CreERT2 transgenic mice (one line shown in Figure 5). We bred the MG-CreERT2 mice with Rosa26 flox-Stop-flox tdTomato reporter mice (MG-CreERT2::R26T) and examined the recombination efficiency of the inducible Cre recombinase. In this analysis, we used R26DT mice (Rosa26 flox-Stop-flox tdTomato reporter mice with constitutive stop codon deletion) as a positive control in which all cells expressed tdTomato fluorescence, while wild-type C57BL/6 mice served as a negative control. Our results indicate that MG-CreERT2 efficiently activated Cre activity in the majority of LSK cells. The vehicle-treated MG-CreERT2::R26T mice and the wild-type negative control mice had no tdTomato-positive cells (Figure 5), indicating the absence of Cre-recombinase activity without prior tamoxifen treatment.

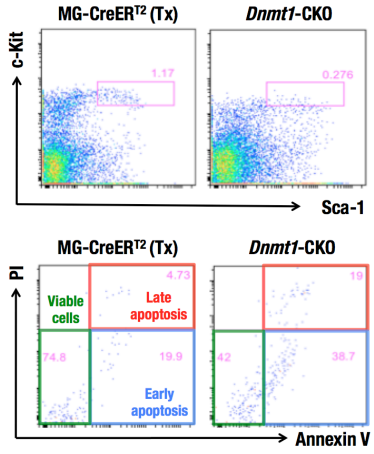

FIG 6. Dnmt1 conditional knockout pheno-copies GATA1 over-expression.

Previously our lab found that Dnmt1 is recruited to the G1MDR and participates in the maintenance of DNA methylation in the *Gata1* locus in HSPCs. Therefore, we attempted to further dissect this mechanism by crossbred the MG-CreERT2 mice with Dnmt1f/f and generated Dnmt1f/f::MG-CreERT2 mice. We refer to these mice as Dnmt1-CKO mice and used them to elucidate the consequences of Dnmt1 deletion in HSPCs. The genetic Dnmt1 ablation caused an increase of Gata1 expression, which recapitulated the HSPC deficiency in the MG-G1 and tamoxifen-treated MG-G1ERT2 mouse models. These results strongly support that Dnmt1 repressed the *Gata1* gene expression.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　5　件）

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〔その他〕
ホームページ等

プレスリリース
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６．研究組織
(1)研究代表者
于　磊　　（Yu Lei）
東北大学大学院・医学系研究科・産学官連携研究員
研究者番号：00760654