

Dynamic regulation of GATA2 in fate determination in hematopoiesis: possible approach to hPSC-derived hematopoietic stem/progenitor cells

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Abstract

GATA2, a principal member of the GATA family, plays important roles in the generation and maintenance of hematopoietic stem/progenitor cells. Among the three mRNA transcripts, the distal first exon of GATA2 (IS exon) is specific for hematopoietic and neuronal cells. GATA2 mutants with abnormal expression are often present in acute myeloid leukemia-related familial diseases and myelodysplastic syndrome, indicating the crucial significance of GATA2 in the proper maintenance of blood system functions. This article offers an overview of the regulation dynamics and function of GATA2 in the generation, proliferation, and function of hematopoietic stem cells in both mouse and human models. We acknowledge the current progress in the cell fate determination mechanism by dynamic GATA2 expression. The gene modification approaches for inspecting the role of GATA2 in definitive hematopoiesis demonstrate the potential for acquiring hPSC-derived hematopoietic stem cells via manipulated GATA2 regulation.

Keywords: GATA2, Hematopoiesis, Stem cells

1. INTRODUCTION

GATA2 has a fundamental impact on the generation and maintenance of hematopoietic stem/progenitor cells (HSPCs). Like other key transcription factors (TFs) such as *FLI1*, *ERG*, *RUNX1*, *SCL*, *LYL1*, and *LMO2*, GATA2 not only marks the initiation of human hematopoietic stem cells (HSCs) but also exerts refined spatial/temporal control during the development and differentiation of HSPCs in various patterns. Despite much accumulated knowledge, its influence on transcriptomic regulation and its subtle yet dynamic expression patterns during hematopoietic development remain largely unexplored. This article summarizes the current data on the role of GATA2 TFs in the regulation of developmental hematopoiesis.

2. STRUCTURE AND PROPERTIES OF GATA2

Human *GATA2* (13,760 kb) is located on chromosome 3¹ (Fig. 1A). The gene is mainly expressed as two different mRNA transcripts of 3484 and 3263 base pairs. A recently identified *GATA2* transcript variant of 3383 bp has been included in the NCBI database (Fig. 1A). *GATA2* mRNA transcription is initiated from two distinct first exons: IS (specific) and IG (general). The distal first exon of *GATA2* (IS), which encodes a protein comprising 480 amino acids (50.5 kDa), is essential for hematopoietic and neuronal cells with significant specificity^{2,3} (Fig. 1A). It has been well documented that the *GATA2* protein-domain function modulating hematopoietic cell fates critically depends on conserved N- and C-zinc finger domains and nonfinger regions, which include two transactivation domains, a nuclear localization signal, and a negative regulatory domain^{4,5} (Fig. 1A).

GATA2 is highly expressed in bone marrow (BM) HSCs and downregulated during lineage commitment,⁶ and its mutations with abnormal expression are often found in acute myeloid leukemia-related familial diseases and myelodysplastic syndrome (Fig. 1C).⁷⁻⁹ The mutation often occurs in the DNA-binding C-finger.¹⁰ The *GATA2* +9.5 kb and -77 kb cis-regulatory elements (also called enhancer) mutations suppress *GATA2* expression.¹¹⁻¹⁴ In contrast, *GATA2* overexpression (OE) in acute myeloid leukemia can predict poor prognosis (Fig. 1C).¹⁵ To date, no distinct *GATA2* mutations have been identified in hematological diseases. However, compared with healthy controls, significantly decreased *GATA2* mRNA expression in CD34-positive cells was observed in patients with aplastic anemia.¹⁶ Taken together,

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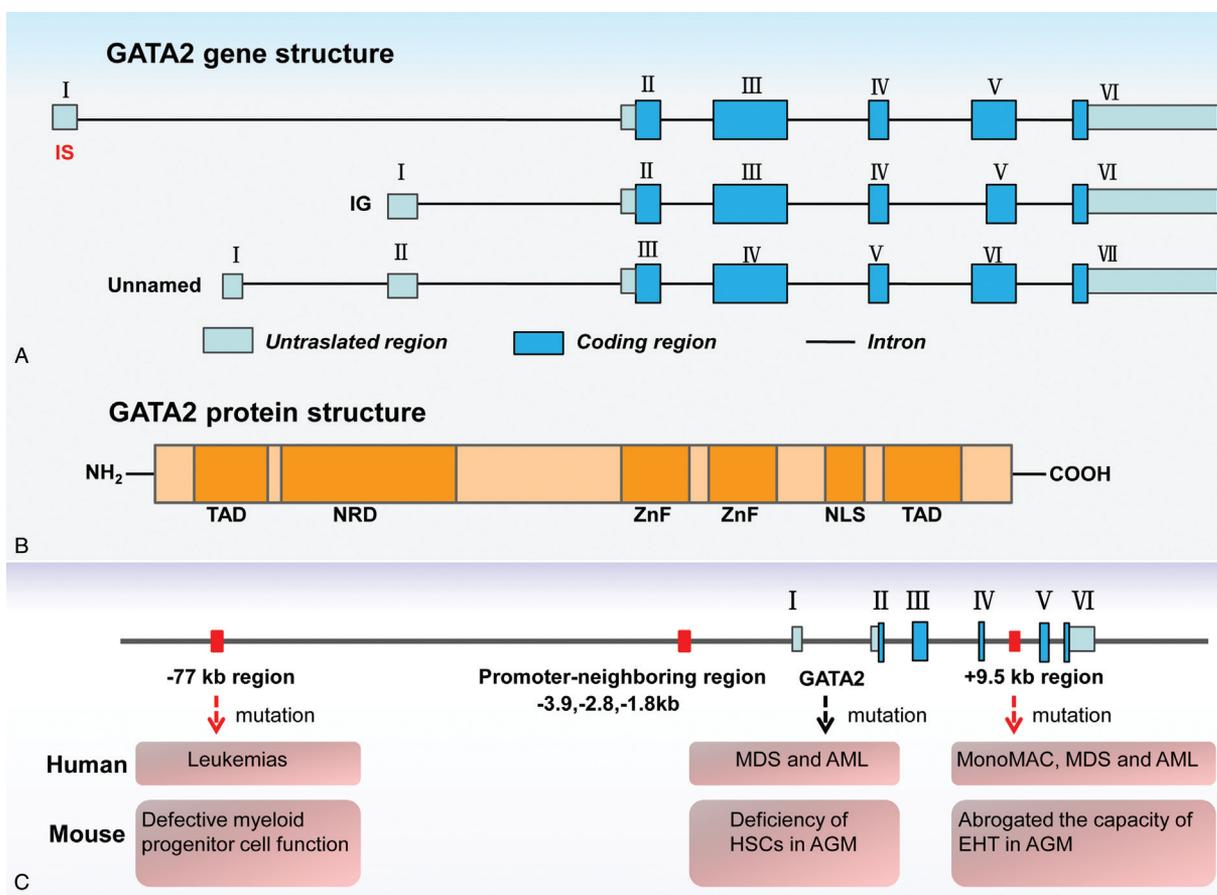


Figure 1. Genomic and protein structure and cis-regulatory elements of GATA2. (A) Schematic representation of human GATA2. The three isoforms IS, IG, and unnamed of human GATA-2 have 6, 6 and 7 exons, respectively. (B) Schematic representation of human GATA2 protein. The encoded protein possesses two transactivation domains (TAD), a negative regulatory domain (NRD), two zinc finger (ZnF) domains, and a nuclear localization signal (NLS). (C) Cis-regulatory elements of GATA2. The -77-kb region, promoter-neighboring region (-3.9, -2.8, and -1.8 kb), and +9.5-kb region are depicted in red. Human and mouse hematopoietic disorders caused by mutations in the cis-regulatory elements and coding region of GATA2 have been indicated.

abnormal GATA2 activity is detrimental during lineage maturation and may lead to hematopoietic disorders.

3. GATA2 PLAYS DISTINCT ROLES AT DIFFERENT STAGES OF HEMATOPOIETIC DEVELOPMENT

GATA2 is initially expressed in the primitive streak, some endothelial cells (ECs) of the dorsal aorta, and vitelline/umbilical arteries of mouse embryos.^{2,17,18} In the course of endothelial-hematopoietic transition (EHT) and later the definitive HSPC formation in the aorta-gonad-mesonephros (AGM) region, GATA2 is expressed in hemogenic endothelium (HE) and intra-aortic hematopoietic cluster cells.¹⁹ These findings indicate that temporal and spatial regulation patterns may be key to demonstrating the specific functions of GATA2. Over the past two decades, various GATA2 mutations have been identified as the causes of inherited and sporadic hematopoietic disorders. The contribution of GATA2 to hematopoiesis in vivo and in vitro has been extensively investigated via genetic engineering. Genotype-phenotype correlations for GATA2 mutations in mice and humans have been summarized in Table 1.

3.1. GATA2 is essential for EHT and HSC generation in the AGM region

Gata^{-/-} embryos die in midgestation when HSCs are generated in the AGM region at embryonic day (E) 10.5²⁰ (Fig. 2A). Gata2 heterozygous mutant (Gata2^{+/-}) embryos with reduced expression of Gata2 are severely affected in the production of early hematopoietic progenitors²⁰ with reduced HSC counts in the AGM region.³³ Conditional knockout with Gata2 in mouse ECs showed deficiency of long-term repopulating HSCs.²¹ Targeted deletion of Gata2 cis-element (+9.5) with reduced Gata2 expression also abrogated the capacity of HE to generate HSC-containing clusters in the AGM region.²² Thus, Gata2 has distinct roles during the different stages of hematopoietic development and is a pivotal regulator of EHT and then HSC generation.

To further understand the requirement for Gata2 in normal hematopoietic development, a mouse model in which an expression impact-free fluorescent reporter for Gata2 (*IRE5-Venus* knock-in gene) was implemented¹⁸ (Fig. 2B). Through vital imaging of single cells in the mouse embryonic aorta (WT and Gata2 heterozygous mutant), the authors found that cell

Table 1**Genotype–phenotype correlations for GATA2 mutations in mice and humans.**

Type of gene manipulation	Species/type	Phenotype	References
Knockout (<i>Gata2</i> ^{-/-})	Mouse/embryos	Fetal liver anemia and died at E10, just before the appearance of the first HSCs	[20]
Conditional knockout	Mouse/ECs	Deficiency of long-term repopulating HSCs	[21]
Knockout (<i>Gata2</i> ^{+/-})	Mouse/embryos	Severely affected the production of early progenitors and greatly reduced the number of HSCs in the AGM region	[20,33]
Targeted deletion of <i>Gata2</i> cis-element (+9.5)	Mouse/embryos	Abrogated the capacity of EHT in AGM	[22]
Targeted deletion of <i>Gata2</i> cis-element(-77)	Mouse/embryos	Defective myeloid progenitor cell function	[11]
<i>Gata2</i> Venus reporter	Mouse/embryos	Cell states during EHT correlate with <i>Gata2</i> reporter expression duration, levels, and pulse periodicity	[18,19]
Knockout (<i>Gata2</i> ^{-/-})	Mouse/ESCs	Defective production of all hematopoietic lineages	[37]
Knockout (<i>Gata2</i> ^{-/-})	Human/ESCs	Reduced EHT during blood differentiation	[38]
Enforced expression	Mouse/bone marrow cells	Blocked both amplification and differentiation of HSCs	[35]
Leaky <i>Gata2</i> overexpression	Mouse/bone marrow cells	Enhanced self-renewal of myeloid progenitors in vitro and blocked lymphoid differentiation	[36]
Inducible <i>GATA2</i> overexpression	Human/CD34 ⁺ cord blood cells	Blocked hematopoietic reconstitution after transplantation	[34]
<i>GATA2</i> w/eGFP reporter	Human/ESCs	<i>GATA2</i> /eGFP ⁺ hESCs cause an increased number of HSPCs	[27]
Dox-induced <i>GATA2</i> overexpression	Human/ESCs	Promotes EHT and maintains HSPCs by inducing cell cycle arrest	[32]
Conditional <i>GATA2</i> expression (<i>IG2</i> ^{-/-} hESCs)	Human/ESCs	Dispensable for specification of HE but promotes EHT	[28]

states during EHT correlate with *Gata2* reporter expression duration, levels (amplitude changes), and pulse periodicity¹⁹ (Fig. 2B), thereby demonstrating a wave-like expression pattern of *Gata2*.

Developmental hematopoiesis has been mainly studied in mouse models, human early hematopoiesis still remains poorly understood. The establishment of human embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs) has enabled new approaches for unveiling the mechanism underlying early hematopoiesis in humans.^{23–25} During the development of hPSCs to HSPCs in vitro, *GATA2* is initially expressed in KDR⁺APLN⁺CD34⁻CD43⁻ definitive mesodermal precursors.²⁶ *GATA2*/eGFP⁺ hESCs give rise to increased numbers of HSPCs.²⁷ *GATA2*^{-/-} hESCs exhibit significant defects in the EHT as well as consequential conversion into HSPCs, whereas the specification of HE remains unaffected.²⁸ By contrast, enforced expression of *GATA2* with *ETV2* or *TAL1* in hPSCs promotes HE with pan-myeloid or erythro-megakaryocytic potential.²⁹ We previously reported an efficient coculture system with AGM-S3 cells that facilitates definitive hematopoiesis from hPSCs in vitro.^{30,31} More recently, we established an hESC line with inducible *GATA2*-OE by doxycycline (Dox) treatment in a PB-based Tet-on system to analyze the functional role of *GATA2* in the early stages of hematopoiesis in humans (Fig. 2E). *GATA2*-OE enhances definitive hematopoiesis by promoting EHT with conserved cooperation of these critical genes for definitive hematopoiesis.³² Thus, *GATA2* is essential for EHT in both mice and humans. The role of *GATA2* in the generation of HE still needs further inspection.

3.2. *GATA2* regulates the functional quality of HSPC fate

Previous studies have demonstrated that the intensity of *GATA2* expression is important in regulating the quantity and function of HSCs.^{33,34} Besides, *GATA2*-OE promotes quiescence

in human cord blood-HSCs, thereby sacrificing their reconstitution capabilities³¹ (Fig. 2D). Enforced expression of *GATA2* also blocks the proliferation and differentiation of murine BM cells³⁵ (Fig. 2C). Low-level enforced expression of *Gata2* in mouse BM cells enhances self-renewal of myeloid progenitors in vitro, blocking lymphoid differentiation.³⁶ Furthermore, HSCs in *GATA2*^{+/-} mice are less competitive than those in wild-type ones in hematopoietic reconstitution posttransplantation.³³ Both enforced and reduced expression of *GATA2* lead to qualitative defects of HSCs (Fig. 2C). *GATA2* appears to elicit a critical dose-dependent effect on the maintenance of HSCs during early and late hematopoiesis. In our system, a dual effect of *GATA2*-OE has been found to promote pre-HSPCs (typically CD34⁺CD43⁺CD45⁻ cells) through the EHT and later arrest hESC-derived HSPCs (CD34⁺CD45⁺) in a quiescent stage via cell cycle regulation (Fig. 2D).

This indicates that the expression duration, level (amplitude changes), and pulse periodicity of *GATA2* must be constrained within a physiological window. *GATA2* is essential for multiple steps during hematopoiesis. It triggers HE to produce HSCs and regulates HSC activity as well as stimulates the proliferation, differentiation, and survival of myeloid progenitors. However, how *GATA2* controls these different processes and to what extent it influences cell fate determination remain elusive.

4. MECHANISM OF CELL FATE DETERMINATION BY DYNAMIC *GATA2* EXPRESSION

In mouse *Gata2*, cis-regulatory elements are located -77, -3.9, -2.8, -1.8, and +9.5 kb from the transcription start site of IS (Fig. 1). Mice lacking the -3.9, -2.8, or -1.8-kb upstream promoter-neighboring regions are born alive without significant hematopoietic abnormalities.^{39,40} Dysfunction of the -77-kb enhancer region decreases *Gata2* expression in myeloiderythroid

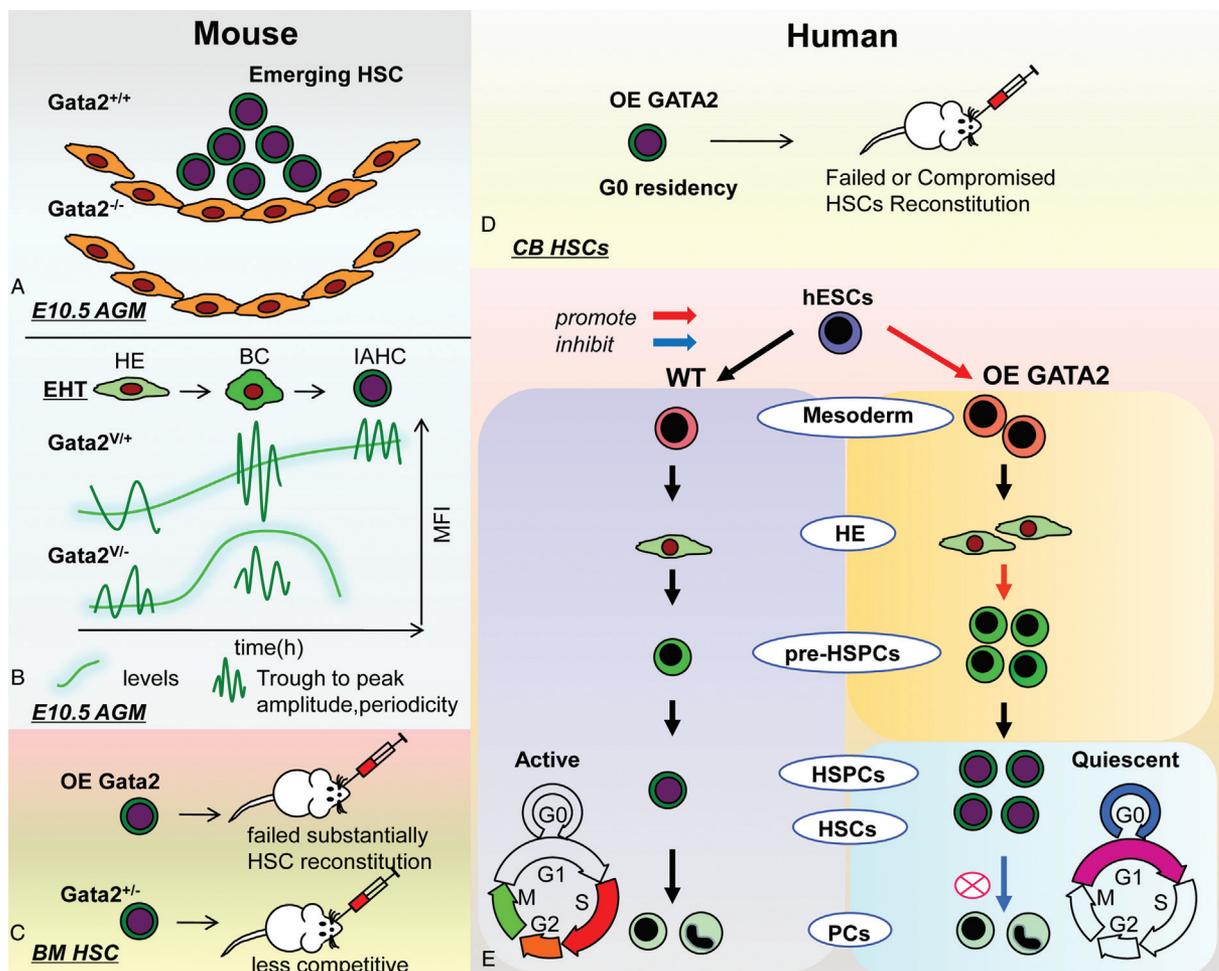


Figure 2. Schematic illustration of the role of abnormal GATA2 expression in mice and humans. (A) Schematic representation of the role of Gata2 knockout in the EHT in the E10.5 mouse AGM region. (B) Model of Gata2 expression dynamics and pulsatile characteristic during EHT in the mouse AGM region at embryonic day (E) 10.5. EHT is accompanied by the transition from HE, and bulging cells emerge from the wall of AGM to intra-aortic hematopoietic cluster cells (IAHCs), which are round cells found in clusters adjacent to the endothelial layer. EHT cell types (top) are shown with accompanying dynamic Gata2 expression changes in EHT directly below. MFI (bright green) and pulse parameters (dark green sinusoids) are shown for $Gata2^{V/+}$ (middle) and $Gata2^{V/-}$ (bottom) EHT subset cells. MFI: mean fluorescent intensity. (C) Schematic illustration of the role of GATA2 overexpression and haploinsufficiency in mouse bone marrow cells. (D and E) Schematic illustration of the role of inducible GATA2 overexpression in human cord blood cells and hESCs.

progenitors but not in HSCs.¹¹ However, the deletion of a cis-element located +9.5-kb downstream of the *Gata2* promoter in mice decreases *Gata2* expression in HE in the AGM region and abrogates EHT.²² The cis-regulatory elements are important in the regulation of Gata2 expression and function in mice, and mutation of the cis-regulatory elements has also been reported in hematopoietic disorders in humans. However, how the cis-regulatory elements regulate GATA2 expression in early hematopoiesis in humans requires further exploration.

Few studies have explored the roles of miRNAs in the GATA2 signaling cascade. Recent reports have shown that upon miR-382-5p overexpression, GATA2 was downregulated in cord blood CD34⁺ HSPCs, causing a significant decrease in megakaryocyte precursor levels with increased granulocyte levels.⁴¹ A heptad of key TFs (FLI1, ERG, GATA2, RUNX1, SCL, LYL1, and LMO2) in human CD34⁺ HSPCs associated with differential microRNA expression was reported.⁴² The GATA2 TF activity is also modulated by posttranslational modifications, such as phosphorylation,^{43–45} sumoylation,⁴⁶ ubiquitination,⁴⁷ and acetylation.⁴⁸ The mechanism of cell fate

determination by dynamic GATA2 expression is complex, suggesting the existence of a multidimensional GATA2-centered network in hematopoiesis regulation.

5. TOOLS FOR INSPECTING THE ROLE OF GATA2 IN HEMATOPOIESIS

Exogenous, conditionally expressed GATA2 or its reporters are routinely used to investigate GATA2 function in mice and humans (Table 2). Various gene modification approaches offer unique advantages for expressing exogenous and endogenous GATA2 for distinguishable and reproducible phenotypes. Lentiviral and retroviral systems were used to express exogenous GATA2 at high levels in mouse and human BM or CB cells.^{31,32} However, potential transgene integration into the promoter/enhancer or gene coding region and the laborious nature of virus production restrict the utility of retroviral and lentiviral vectors.⁴⁹ Compared with retroviral or lentiviral systems, the PiggyBac system presents a promising option for the nonviral genetic engineering of hPSCs. The system comprises a PiggyBac

Table 2**Tools used in the modulation of GATA2 expression in mice and humans.**

Levels of GATA2 expression	Tools for gene modulation	Species/type	References
Enforced	Retrovirus	Mouse/bone marrow cells	[35]
Low-level enforced	Retrovirus	Mouse/bone marrow cells	[36]
Inducible enforced	Lentivirus	Human/CD34 ⁺ cord blood cells	[34]
Inducible enforced	PiggyBac transposon system	Human/ESCs	[32]
None	HR	Mouse/embryos	[20]
None	HR	Mouse/endothelial cells	[21]
Reduced	HR	Mouse/embryos	[20,33,22]
Normal	HR	Mouse/embryos	[18,19]
None	HR	Mouse/ESCs	[37]
None	TALEN	Human/ESCs	[38]
Normal	TALEN	Human/ESCs	[27]
Conditional overexpression of GATA2	PiggyBac transposon system and CRISPR/Cas9	Human/ESCs	[28]

CRISPR/Cas9 = CRISPR/Cas9 system, HR = homologous recombination, PB transposon = PiggyBac transposon system, TALENs = transcription activator-like effector nucleases

transposon vector and a transposase vector, which transiently expresses the transposase. Thus far, we have established hESC lines utilizing such a system with a Dox induction to analyze the functional roles of GATA2 in cell subsets (mesoderm, EHT, HSPCs, and other hematopoietic cells)²⁹ (*data not shown*). The relative expression of exogenous and endogenous GATA2 could also be distinctively detected by qRT-PCR.

Conventional gene-targeting methods via homologous recombination (HR) have been extensively used to specifically alter genes in mouse models. This approach has proven to be invaluable in its use in mouse ESCs to generate *Gata2* knock-out, knock-down, and knock-in mice. A series of *Gata2*^{-/-}, *Gata2*^{+/-}, and site-specific *Gata2*^{-/-} mice was created through HR (Table 2); a *Gata2Venus* reporter model was also created with unperturbed *Gata2* expression to examine its transcriptome profile and hematopoietic functions.^{18,19}

Nevertheless, the conventional HR method presents low efficiency and is laborious due to the requirement of targeting constructs with long homology arms and multiple drug selection rounds to isolate desired clones, raising challenges in hPSC applications.⁵⁰ There is a need for improved efficacy in gene targeting for the manipulation of hPSCs. The novel genome-editing technology, also known as genome engineering, seeks to meet this demand by offering superior efficiency and specificity, ranging from single-nucleotide modifications to whole-gene additions or deletions. Transcription activator-like effector nucleases (TALENs) have been used to knockout GATA2 or knock-in eGFP-GATA2 reporter in hESCs.^{24,38} The CRISPR-Cas9 and PiggyBac transposon systems have been used in the conditional manipulation of GATA2 expression (IG2^{-/-} hESCs).²⁵ The PiggyBac transposon system has been used to engineer an hESC line (H1) carrying a DOX-inducible GATA2 transgene. The CRISPR/Cas9 system has also been used to knockout endogenous GATA2 with targeted guide RNA sequences around exons 2 and 5.²⁵ Hence, the emergence of the novel genome-editing technology has made it much easier to generate and investigate human cellular disease models with even greater speed and efficiency.

In further studies, a combined cocktail of GATA2 and other markers coexpressed during the development of HSPCs, such as KDR, CD34, CD43, and CD45, may be sought. The parallel introduction of fluorescence reporters in both mouse- and hPSC-derived cells may be used to fully elucidate the role of GATA2 in hematopoiesis.

6. IMPLICATIONS AND PERSPECTIVES

Several decades of research on the GATA2 protein family has provided a wealth of molecular and biological information concerning their roles in normal hematopoiesis; however, the phenotypes of these mouse models do not always correlate with the human phenotypes. However, analyses of mouse models have provided important insights for guiding human case analyses and have increased the understanding of the pathophysiological mechanisms underlying human diseases.

It is difficult to assess how the expression levels of GATA2 in engineered hemogenic cells and their transcriptome profiles resemble those of the HSCs in the AGM region, fetal liver, and BM under in vivo physiological circumstances. Realizing that it might not matter if the GATA2 expression is high to achieve the goal of generating multipotent hematopoietic cells, this might be an important parameter, especially given the failure thus far of research groups to demonstrate long-term repopulating activity of HSC-like cells induced by GATA2 in hESCs. The mechanisms via which GATA2 regulates the generation and maintenance of HSCs at different developmental stages must be further elucidated.

Using the newly implemented single-cell analysis method, a time-lapse image of single cells can be constructed, resolving the GATA2-expressing hematopoietic cell populations located in AGM, fetal liver, and BM. Such information can further map out the dynamic expression profile of GATA2 and its related TF assemblies and reshape the epigenetic landscape in the process. Transcriptomic and proteomic signatures acquired are expected to uncover the mechanisms underlying the inherent instability of hematopoietic cells. Taken together, these insights into GATA2 proteins may ultimately generate novel approaches to acquiring HSCs from hPSCs for both academic and clinical applications.

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