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Chapter 2

Leukemogenesis in Down syndrome

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Additional information is available at the end of the chapter

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Abstract

The incidence of leukemia is higher in Down syndrome children than that in the general population, while the risk of solid tumors is significantly reduced in Down syndrome. Recent studies utilizing mouse models have shown that distinct mechanisms caused by the elevated dosage of multiple genes is implicated in the protection from tumor progression depending on the type of solid neoplasm. In contrast, increased incidence of mutation in the several specific genes is reported as a cause of the onset of leukemias. Especially, acquired mutations in the *GATA1* gene are associated with leukemogenesis of megakaryoblastic leukemia (AMKL) and transient myeloproliferative disorder (TMD) related to Down syndrome. The mutations are clustered in the region corresponding to the N-terminal domain of GATA1 and result in the production of the short form of GATA1 (GATA1-S), which utilizes Met84 as an alternative translation initiation codon. Efforts producing mouse models of Down TMD and AMKL have been undertaken, as these models seem to provide important insights into the pathogenesis of multistep leukemogenesis. Concomitantly, the function of GATA1 has been examined extensively, and the analyses present a prototype for the study of lineage-restricted transcription factors that play an essential role for the differentiation, proliferation, and apoptosis of erythroid cells, megakaryocytes, mast cells, and eosinophils. In this chapter, we will summarize recent progress in the studies of leukemias that occur in Down syndrome, especially in relation to GATA1 mutations.

Keywords: Acute megakaryoblastic leukemia, Acute lymphoblastic leukemia, Transient myeloproliferative disorder, GATA1, Down syndrome

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1. Introduction

The risk of solid tumors is significantly reduced in Down syndrome (DS) patients compared with the general population [1-3]. Studies utilizing mouse models have shown that distinct mechanisms caused by the elevated dosage of multiple genes are implicated in the protection from tumor progression depending on the type of solid neoplasm. In contrast, DS children have an increased risk of developing leukemias. Specifically, acute megakaryoblastic leukemia (AMKL) and acute lymphoblastic leukemia (ALL) are approximately 500 and 10-50 times more prevalent in children with DS compared to healthy controls, respectively [4-6].

Recent analyses have uncovered that a distinct pattern of acquired genetic mutations is implicated in the leukemogenesis restricted to DS children. In particular, acquired mutations in the *GATA1* gene are associated with leukemogenesis of DS-associated AMKL and transient myeloproliferative disorder (TMD), a preleukemic disorder unique to neonates with DS. The mutations are clustered in the region corresponding to the N-terminal domain of GATA1 and result in the production of a short form of GATA1 (GATA1-S), which utilizes Met84 as an alternative translation initiation codon. The function of GATA1 has been studied extensively, and the analyses present a prototype for the study of lineage-restricted transcription factors that play essential roles in the differentiation, proliferation, and apoptosis of erythroid cells, megakaryocytes, mast cells, and eosinophils. In this chapter, we summarize recent progress in the research on leukemias related to DS. Research of mouse models of DS TMD and AMKL has been undertaken, as these models seem to provide important insights into the pathogenesis of multistep leukemogenesis.

2. Unique features of leukemias associated with Down syndrome

There are substantial biological differences between the leukemias that occur in DS and non-DS children. It becomes increasingly clear that molecular mechanisms based on trisomy 21 affect not only the high incidence of leukemias but also the characteristic features of DS-associated leukemias.

2.1. DS-associated AMKL

Approximately 5-30% of DS babies are born with a leukocytosis with an erythroid-megakaryocytic immunophenotype. The severity of the clinical course varies with each case, from asymptomatic cases likely to have been considered peaceful births to severe cases resulting in critical injuries with blast cell infiltration of the organs. In severe cases, death from multiorgan failure or hepatic fibrosis is possible, requiring intensive care for the babies. Except for the most severe cases, symptoms usually resolve naturally (Figure 1). Therefore, DS-related leukocytosis is referred to as transient abnormal myelopoiesis (TAM) or transient myeloproliferative disorder (TMD). Patients suffering from TAM spontaneously achieve complete remission by 6 months of age. While the mechanisms of this spontaneous remission are still under investigation, one recent report showed that type I interferon signaling activated in bone marrow appears to attenuate the hyper-proliferation of megakaryocytes caused by the GATA1-S mutation [7].

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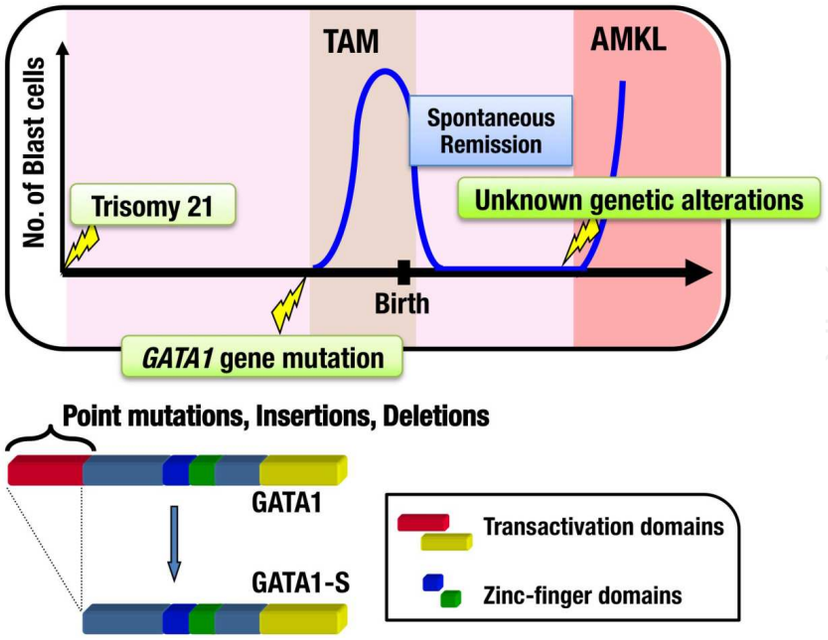


Figure 1. Multistep leukemogenesis of DS-associated AMKL. Megakaryocytic progenitors that have acquired various types of *GATA1* gene mutations eventually converge to GATA1-S, which develops TMD in DS fetus. The hyperproliferation phenotype of TAM blasts is canceled after birth. However, unknown genetic alterations transform the residual blasts to genuine leukemia.

After several years of asymptomatic periods, approximately 20% of DS children with a history of TAM develop genuine AMKL requiring intensive chemotherapy (Figure 1). It has been hypothesized that every DS child first diagnosed with AMKL has a history of asymptomatic TAM at birth. As a result, the incidence of AMKL in DS children is more than 500 times higher than in children without trisomy 21. Thus, this characteristic feature of DS-associated AMKL represents a potentially tractable model of multistep leukemogenesis. Chemotherapy in children with DS-associated AMKL shows a high cure rate. Therefore, the outcome of DS children with AMKL is statistically better than that of AMKL patents without trisomy 21 [8], probably due to the unique biological characteristics of blast cells. The molecular mechanisms of these remarkable differences remain as yet unknown.

In 2001, it was discovered that somatic mutations on the *GATA1* gene are frequently found in AMKL cases occurring in DS children [9]. Supporting evidence has appeared following the release of a paper describing the *GATA1* gene mutations found in TAM and DS-associated AMKL cases (Figure 1). Of note, the mutations are clustered in the region corresponding to the N-terminal domain of GATA1 and result in the production of the short form of GATA1 (GATA1-S) utilizing Met84 as an alternative translation initiation codon (Figure 1). A clinical study utilizing Guthrie blood shows that the *GATA1* gene mutation is found in the 3.8% of DS babies who are not diagnosed with TAM at their birth [10]. In some non-DS AMKL cases with the *GATA1* gene mutation, leukemic blasts originate from the cells that have somatically acquired trisomy 21 during myeloid development [11]. Similar cases are also observed in cells carrying trisomy 21 in mosaic models [12], suggesting that a cell-autonomous program provoked by the GATA1 mutation and trisomy 21 contributes to the onset of TAM. Relatively

few cases of AMKL harboring mutations in the *GATA1* gene exist in the absence of trisomy 21 [13, 14]. Therefore, the pathogenesis of TAM seems to be closely associated with GATA1 dysfunction in combination with the impact provided by the extra chromosome 21. One of the important remaining questions is how DS children frequently acquire the *GATA1* gene mutation.

Sequential surveys of cases of TAM-AMKL have revealed that the type of *GATA1* gene mutations detected in AMKL blasts is frequently identical to that found in TAM blasts in each individual case, suggesting that AMKL develops in the TAM blasts. It seems plausible that TAM blasts survive during an asymptomatic period and some of the blasts receive additional hits and become genuine leukemic stem cell (Figure 1). Indeed, exome sequencings of TAM and AMKL blasts have revealed that the TAM blasts from one case have a singular *GATA1* gene mutation, while multiple mutations in the genes encoding for the cohesin components and epigenetic regulators are accumulated in the AMKL blasts in addition to the original mutation in the *GATA1* gene [15].

2.2. DS-associated ALL

Acute lymphoblastic leukemia (ALL) is one of the most common malignant diseases in children, and DS children have a higher incidence of this disease than non-DS children. In contrast to the DS-associated AMKL cases, the prognosis of DS-associated ALL is worse than ALL without DS. This may be because DS children are prone to suffer from significant toxicity of chemotherapy, as DS-associated ALL blasts retain high resistance against conventional chemotherapy regimens [16-18]. Intrachromosomal amplification of chromosome 21 (iAMP21) is also recurrently found in pediatric B cell leukemia, and this finding is considered to be an adverse prognostic factor [19, 20]. It has been demonstrated that target genes of the polycom repressor complex 2 (PRC2) are upregulated in both DS-associated ALL and ALL with iAMP21 [21], indicating that an epigenetic regulation provoked by trisomy 21 is a key mediator of the pathogenesis of this type of leukemia.

Mutations in the developmental genes for B-cell and T-cell lineages are often implicated in the pathogenesis of ALL, irrespective of the presence of Down syndrome. In addition to these genes, the incidence of mutations on genes involving the JAK/STAT pathway and RAS signaling have been found to be increased in DS-associated ALL [22-24], suggesting that molecular changes provided by these mutations may be the reason for the poor survival rate in children with DS-associated ALL.

3. Hematopoiesis in Down syndrome

It has been shown that hematopoietic homeostasis is significantly disturbed in DS fetus [25]. For example, frequencies of hematopoietic stem cells and megakaryocyte-erythroid progenitors are markedly increased showing high clonogenic characteristics, whereas that of granulocyte-macrophage progenitors is reduced [25-27]. In addition, committed pre-proB lymphoid and proB lymphoid progenitors are markedly reduced [25, 26]. This hematological abnormal-

ity may be a predominant cause of AMKL and ALL in DS babies. While the hematological abnormalities observed in newborns and infants with DS vary case by case, the following abnormalities are often observed: macrocytosis, neutrophilia, thrombocytopenia, and polycythemia [28-30]. In contrast to the high risk of leukemias in the pediatric period, leukemias are no longer a common cause of death in adults with DS [31]. Instead, macrocytosis and leucopenia are often observed in adult DS cases, leading to the early onset of myelodysplastic syndrome and bone marrow failure [32].

Strictly speaking, DS is defined by the possession of three copies of DS critical region (DSCR) of human chromosome 21 instead of two. However, the expression of genes located on chromosome 21 or the DSCR in DS patients is not always increased to 1.5-fold that of healthy controls. Depending on the expression levels, genes on human chromosome 21 or DSCR are divided into three categories: *i*) overexpressed genes, *ii*) genes expressed comparable to those in diploid healthy controls, and *iii*) genes whose levels are varied in individuals with DS. It has been reported that the frequency of genes overexpressed (type *i*) in B lymphocytes of DS patients is 22-39%, and the set of overexpressed genes in B lymphocytes differs from that found in fibroblasts [33, 34]. Therefore, it seems likely that the genes that proportionally increased due to the gene-dosage effect contribute to the common characteristic feature of DS, while the genes whose expression is varied in individuals determine the conditions of the individuals. In addition, microRNAs may be involved in the determination of DS disease types [35]. Expressional imbalances of chromosome 21-derived microRNAs may contribute to the diversified symptoms in DS patients.

To investigate the contribution of trisomy 21, three groups have independently established iPS (induced pluripotent stem) cells originated from DS patients and analyzed the hematopoietic differentiation. When iPS cells are cultured under the condition of primitive hematopoiesis (the first phase of hematopoiesis in yolk sacs), erythropoiesis is enhanced and myelopoiesis is reduced, while megakaryocytes are normally produced [36]. In contrast, when cultured under the condition of preferentially differentiating into fetal liver–derived definitive hematopoietic cells (the second phase of hematopoiesis in the fetal liver), iPS cells with trisomy 21 show increased multilineage colony-forming potential, and the number of cells with a myeloid and erythroid bipotential phenotype is increased [37]. However, trisomic iPS cells show no difference from isomic iPS cells when they are cultured in a condition suitable to generate erythroblast co-expressing embryonic and fetal globin genes [38]. Thus, the iPS studies suggest that the influence of one extra chromosome 21 to hematopoiesis is altered depending on the hematopoietic microenvironment.

Hematopoietic phenotypes have been examined in multiple lines of DS-model mice. Tc1 mice harbor an aneuploidy, carrying freely segregating human chromosome 21. The Tc1 mice show macrocytic anemia and an increased number of megakaryocytes with extramedullary hematopoiesis in the elderly. On the contrary, significant changes in frequencies of megakaryocytes, erythroid, and myeloid progenitors were not observed in the fetal liver [39]. Ts16 mice were generated by crossing mice with Robertsonian translocation Rb (14;16) and Rb (9;16), resulting in animals trisomic for mouse chromosome 16 (synthetic of human chromosome 21). Ts16 mice show increased erythropoiesis and reduced myelopoiesis during the embryonic period [40],

but defects in hematopoiesis after the neonatal and infantile periods are uncertain, as Ts16 mice do not survive the postnatal period.

Ts65Dn, Tc1Cje, and Ts1Rh mice have been established as lines of euploid DS-model mice bearing a segmented region of mouse chromosome 16 containing 104, 81, and 33 genes, respectively. The Ts65Dn mice suffer from macrocytic anemia with defects of stem cell function and progressive myeloproliferative diseases [41]. In contrast, erythropoiesis is disturbed in Tc1Cje mice, but the mice never develop thrombocytosis or myeloproliferative diseases [42]. Hematopoietic analyses have been performed in Ts1Rhr mice, which are trisomic for the mouse equivalent of the hypothetical human DSCR; these mice show anemia and thrombocytosis in adulthood. Bone marrow cells of the mice preferentially differentiate toward the granulo-monocyte pathway, while the number of B cell progenitors is reduced [21, 43]. During the embryonic stage, hematological abnormalities are not found in Ts1Rhr mice, except for a significant increase in the hematopoietic stem cell population. Although each line of DS-model mice shows partially overlapping hematological phenotypes with those observed in patients with DS, none of the mice develop leukemia or acquire the *Gata1* gene mutation.

4. *GATA1* gene mutation in TAM and DS-associated AMKL

GATA1 is a founding member of the GATA family transcription factors that recognize consensus GATA binding motif. In mammals, the hematopoietic GATA subfamily is composed of GATA1, GATA2, and GATA3, which are expressed in erythroid and megakaryocytic cells, hematopoietic stem and progenitor cells, and T cells, respectively [44]. The expressions of GATA1 and GATA2 are partially overlapping, and these two GATA factors regulate the expression of each other. In early erythroid-megakaryocytic progenitors, GATA2 initiates *GATA1* gene expression, while GATA1 downregulates *GATA2* gene expression together with activation of its own gene expression [45]. We refer to this network regulation of GATA2 and GATA1 as GATA factor switching [45]. In addition, GATA1 and GATA2 share, at least in part, their target genes [46]. Consequently, GATA1 and GATA2 competitively or redundantly work for the expressions of their target genes dependent on the gene properties. Therefore, the functional balance of GATA1 and GATA2 is important for the maintenance of hematopoietic homeostasis.

The *GATA1* gene is composed of one untranslated first exon and five translated exons (Figure 2). The translation initiation codon located in the second exon is usually utilized to produce the full length of GATA1. Interestingly, an alternative splicing variant caused by the skipping of the second exon has been noticed in healthy humans, producing a short form of GATA1 protein lacking amino (N)-terminus 83 amino acids, which is identical to GATA1-S, utilizing an alternative translation initiation codon located in the third exon (Figure 2) [47]. In the case of mice, a short isoform of GATA1, a mouse ortholog of human GATA1-S, is also generated by the use of an alternative translation initiation codon in a single mRNA shared with the full-length of GATA1 [48]. Thus, two forms of GATA1 protein, GATA1 and GATA1-S, are both present simultaneously in healthy humans and mice, although the physiological roles of the GATA1-S isoform remain to be clarified.

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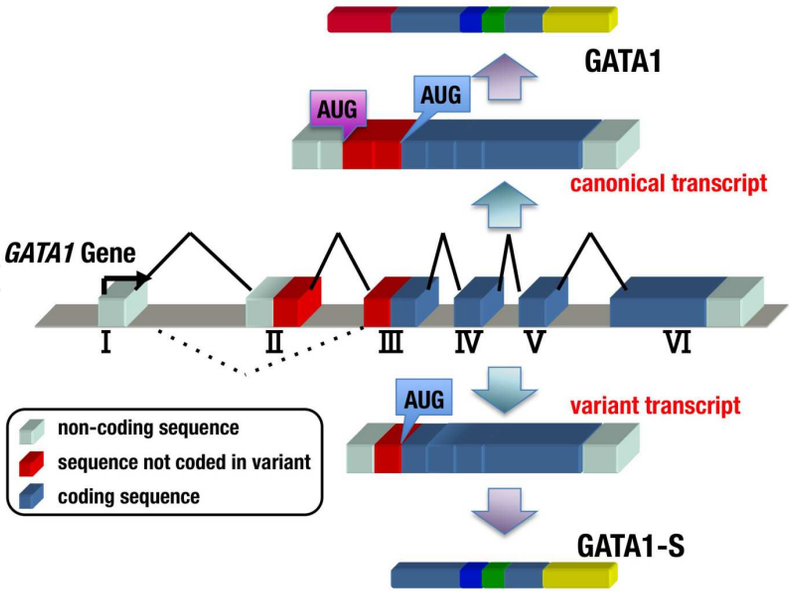


Figure 2. ***GATA1*** **gene construction and splicing variants.***GATA1* gene is composed of an untranslated first exon and five translated exons. Full-length GATA1 is produced utilizing a translation initiation codon located in the second exon (upper panels). On the other hand, the GATA1-S variant is produced based on an alternative splicing variant, skipping the second exon and utilizing a methionine codon AUG corresponding to the 84th amino acid of full length of GATA1 (lower panels). Consequently, GATA1-S lacks the N-terminus 83 amino acids.

The *GATA1* mutations in TAM cases are clustered in the second exon of the *GATA1* gene, leading to the production of frame-shift misincorporation with the premature translation termination codon. Alternatively, mutations inducing pathological skipping of the second exon have also been reported [49]. Thus, the GATA1-S, but not the full-length GATA1, is exclusively produced in the TAM/AMKL blasts, indicating that aberrant function of GATA1-S in the absence of the expression of the full-length GATA1, rather than simple loss-of-GATA1-function, is involved in the onset of TAM.

GATA1 has four functional domains, two transactivation domains in the N- and carboxy (C)-terminal regions and two zinc-finger domains in the middle of body, which are important for the interaction with DNA and multiple cofactors (Figure 3A). Conventional cell-based luciferase reporter assays show that transactivation activity of GATA1-S is reduced into 30% of the full-length of GATA1, which is supported by the function of the C-terminal domain [50]. Functional analyses in mice *in vivo* indicate that the N-terminal and C-terminal transactivation domains differentially and cooperatively contribute to gene expression, depending on the property of GATA1 target genes. GATA1 target genes can be divided into three categories: the first group of genes requires only the N-terminal domain, the second group of genes requires only the C-terminal domain, and the third group of genes requires both domains [50]. The expression of only GATA1-S leads to the imbalance of GATA1 target gene expression, which may be implicated in the pathogenesis of TAM.

Importantly, provability of transformation into AMKL inversely correlates with the expression level of GATA1-S, and the latter is heavily dependent on the type of mutations in *GATA1* gene [48]. In the case of the mutations producing high amounts of GATA1-S, the incidence of AMKL

is relatively low. However, such cases frequently suffer from high blast counts in the peripheral blood and require chemotherapy to prevent organ infiltration with the blasts. In contrast, patients with low amounts of GATA1-S frequently develop AMKL. Thus, the expression levels of GATA1-S influence leukemia progression and disease status.

Recently, two types of internally deleted (ID)-type GATA1 proteins were reported as a cause of TAM (Figure 3B) [51]. In the blasts of these cases, aberrant transcripts are produced due to splicing mutations in the *GATA1*, and, consequently, GATA1 proteins lacking amino acid residues 77-119 or 74-88 are produced. The N-terminal end of GATA1 is retained in the newly identified ID-type GATA1 mutants, while retinoblastoma protein (Rb)-binding motif LXCXE located around the Met84 is commonly deleted in GATA1-S and the ID-type GATA1 mutants (Figure 3B). Because binding potential with the Rb protein is important for the GATA1 functions of controlling cell proliferation and promoting erythroid differentiation [52], loss of the interaction with Rb protein may contribute, at least in part, to the GATA1 dysfunction leading to the pathological process of TAM.

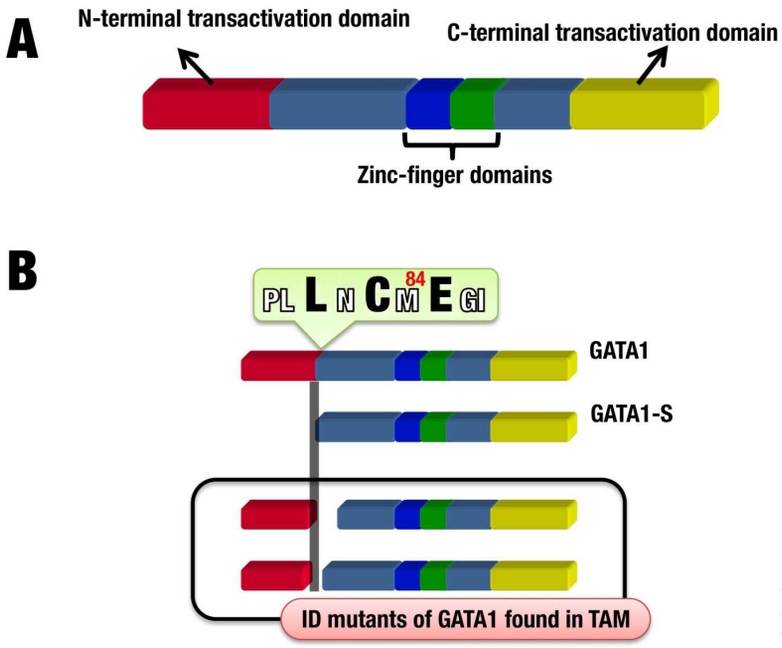


Figure 3. Rb-binding motif is commonly deleted in GATA1-S and the ID-type GATA1 mutants. A: domain structure of GATA1. Of note is that a pRb-binding motif LxCxE (aa 81-85) is commonly in wild-type GATA1. B: when the pRb-binding motif is eliminated in the GATA1-S and ID-type GATA1, the loss-of-function of the LxCxE motif appears to contribute to the TAM genesis.

5. Leukemogenesis caused by GATA1 deficiency

GATA1/*Gata1* gene is located on the X chromosome in humans and mice. Hemizygous male mice with the *GATA1-null* germline mutation die *in utero* due to insufficient primitive

erythropoiesis [53]. CFU-Meg (colony-forming unit-megakaryocyte) is significantly reduced in the yolk sac of *Gata1*-null embryos, indicating that GATA1 is indispensable for the primitive erythropoiesis and megakaryopoiesis. Independently, the *GATA1-knockdown* allele has also been constructed by inserting a neomycin resistance cassette just upstream from the first exon [54]. In this strain of mice, the strong promoter activity within the neomycin cassette directly interferes with the expression of the *Gata1* gene to approximately 5% of the wild-type level [54]. Hemizygous *Gata1-knockdown* mice also die *in utero*, showing a similar phenotype to the *Gata1-null* mice. These results indicate that 5% of the GATA1 level cannot support primitive erythropoiesis.

These two lines of *Gata1*-deficient mice have been maintained through heterozygous female mice. In female mice carrying the *Gata1* mutations, two types of hematopoietic progenitor cells are developed depending on the X chromosome inactivation. Hematopoietic progenitors with inactivated wild-type X chromosome and activated X chromosome with the *Gata1* mutation cannot differentiate into mature erythroid and megakaryocytic cells because of the defect in GATA1 function. In contrast, hematopoietic progenitors in which wild-type X chromosome is activated (and X chromosome with *Gata1* mutation is inactivated) differentiate normally to produce erythrocytes and platelets. Therefore, while *Gata1-null* and *Gata1-knockdown* heterozygous female embryos are anemic, these mice are born alive and grow normally.

However, interestingly, the female *Gata1-knockdown* mice frequently develop leukemia, while female *Gata1-null* mice never develop leukemia and have a normal life expectancy [55]. Characterization of leukemia cells shows that the cells are positive for c-Kit (a stem cell factor receptor) and CD71 (a transferrin receptor) antibodies but negative against the Ter119 (a molecule associated with glycophorin A) antibody. Further analyses have revealed that the leukemia is provoked by the residual 5% GATA1 in the immature erythroid progenitor cells carrying the activated *Gata1-knockdown* allele. In contrast, the progenitor cells with the activated *Gata1-null* allele never commit to leukemogenesis.

Because simple GATA1-deficiency causes a lethal embryonic phenotype, the roles of GATA1 in adult hematopoiesis are investigated using mice with conditional *Gata1* gene ablation. Adult *Gata1-null* mice produced by a conditional deletion of whole coding exons suffer from severe anemia and thrombocytopenia [56]. These mice lack erythroid progenitors, showing a phenotype resembling that of pure red cell aplasia. These results unequivocally indicate that GATA1 is required for erythroid commitment and erythropoiesis in the early stage of hematopoiesis. In addition, well-lobulated mature megakaryocytes that are positive for acetylcholine esterase (AchE) staining are accumulated in the hematopoietic organs of conditional *Gata1-null* mice, indicating that GATA1 is dispensable from the early stage of megakaryopoiesis and megakaryocyte development to matured polyploid megakaryocytes [56].

An additional line of GATA1-deficient mice (referred to as $Gata1^{\Delta IE}$) has been produced by a conditional deletion of the promoter/first exon of the *Gata1* gene [57]. The conditional adult $Gata1^{\Delta IE}$ mice also suffer from anemia. However, unlike the conditional *Gata1-null* mice, immature erythroid progenitors lacking terminal maturation potential to produce enucleated erythrocytes are accumulated in the hematopoietic organs of conditional $Gata1^{\Delta IE}$ mice. Also in these conditional $Gata1^{\Delta IE}$ mice, a small amount of aberrant *Gata1* transcript is produced in

the erythroid progenitors utilizing alternative first exons located in the first and the second introns. These transcripts produce a small amount of GATA1-S, which supports the erythroid commitment of hematopoietic progenitors, but the cells fail to complete terminal erythroid maturation. In contrast, these aberrant transcripts are not produced in the megakaryocytes, and the megakaryocytic phenotype of the conditional *Gata1*$^{\Delta IE}$ mice is identical to that of the conditional *Gata1*-null mice.

We have generated germline *Gata1*$^{\Delta IE}$ mice by crossing the original flox mice with general Cre-expressing mice. It is worth noting that heterozygous female mice with the germline *Gata1*$^{\Delta IE}$ allele are prone to developing erythroleukemia, similar to the female *Gata1-knockdown* mice [58]. Thus, the impacts of changes in the expression level of GATA1 lead to the progression to leukemia in the mice, while the consequences also affect the erythroid differentiation differentially.

6. Mouse models of TMD and DS-associated AMKL

A transgenic complementation rescue approach has been successfully applied for the investigation of GATA1 function *in vivo*. Expression of full-length GATA1 under the transcriptional regulatory influences of *Gata1* recapitulates the GATA1 function *in vivo* and rescues various GATA1-deficient mice nicely from embryonic lethality [59]. Because transgene-derived mutant GATA1 is exclusively expressed in the rescued mice, one can reasonably expect that the phenotypes observed in the rescued mice occur due to the mutation.

Exploiting this rescue strategy, we have established transgenic lines of mice expressing GATA1-S. Transgenic expression of GATA1-S rescues the GATA1-deficient males from embryonic lethality [60]. Strikingly, immature megakaryocytes are accumulated in the fetal livers of the rescued mice, but this phenotype disappears after birth [61], indicating that the simple GATA1-S mutation provokes TMD-like phenotype in mice regardless of the presence of disomy or trisomy of chromosome 16 (the equivalent of human chromosome 21). In a colony-forming assay, progenitors with the GATA1-S mutation produced multiple progenies and generated enormously large colonies, consisting of innumerable immature megakaryocytes that were faintly positive for the AchE staining. In this assay, the number of CFU-Megs in the rescued embryos was equivalent to that in the wild-type embryos. Thus, GATA1-S failed to control proliferation and differentiation during the megakaryocyte differentiation process, resulting in an uncontrolled growth of immature megakaryocytes. It should be noted that TAM appears to be generated by single or a couple of hematopoietic progenitors acquiring the GATA1-S mutation, whereas in the rescued mice, all progenitors express GATA1-S. These observations suggest that the condition provided by trisomy 21 may lead to the onset of TAM by exploiting two pathways: one increases the frequency of GATA1-S mutation, while the other activates cellular mechanisms that magnify the impacts of the GATA1-S mutation.

One of the strong advantages of the transgenic complementation rescue assay is that levels of transgene-derived GATA1-S can be controlled. Because the expression levels of transgene-derived transcript vary between transgenic mouse lines depending on the integrated positions

and/or copy numbers of the transgene, transgenic mouse lines expressing high-level GATA1-S and low-level GATA1-S have been used for the rescue analyses. The mice expressing high levels of GATA1-S never develop leukemia, whereas mice expressing low levels of GATA1-S are prone to developing leukemia. The leukemic cells have biphenotypic characteristics of erythroid and megakaryocyte lineages, closely resembling the human DS-associated AMKL (our unpublished observation). Thus, the expression levels of GATA1-S appear to be reflected in the transformation process of TAM blasts to genuine leukemia, consistent with the case of leukemogenesis in the erythroid progenitors of *Gata1-knockdown* mice caused by GATA1-deficiency.

Mice expressing GATA1 lacking N-terminal 63 amino acids have been established by gene targeting [62]. This line of mice showed transient hyper-proliferation of megakaryocytes in the fetal livers during the early stage of development, whereas the phenotype of the megakaryocytes in the later embryonic stages was close to normal. Because this mutant GATA1 molecule preserves the consensus Rb-binding motif, the effect of GATA1 mutation may be reduced. Another line of mice has been established by second exon deletion (*Gata1*$^{\Delta e2}$), and, consequently, only GATA-S is expressed in the *Gata1*$^{\Delta e2}$ mice [62]. This line of mice also shows transient hyper-proliferation of megakaryocytes during the early embryonic stage, similar to the GATA1 mutants lacking N-terminal 63 amino acids. In addition, the megakaryocyte progenitors derived from bone marrow of the adult *Gata1*$^{\Delta e2}$ mice are hyper-proliferative and formed significantly larger colonies than those in wild-type mice [7]. While the mechanism underlying the difference in the phenotypes of the rescued mice (hyper-proliferation of megakaryocytic progenitors until the perinatal stage [61]) and the *Gata1*$^{\Delta e2}$ mice (hyper-proliferation of megakaryocytic progenitors only in the early embryonic stages [62]) is unclear at present, these data congruently support the contention that the GATA1-S mutation alone gives rise to the hyper-proliferation of megakaryocytic progenitors in fetal mouse livers.

By crossing *Gata1*$^{\Delta e2}$ mice with DS-model lines of mice, *i.e.,* Tc1 and Ts1Rh, the contributions of GATA1-S mutation in combination with the trisomy of chromosome 21 to the leukemogenesis have been analyzed. The frequency of CFU-Megs in fetal livers is increased by the combination of *Gata1*$^{\Delta e2}$ and Tc1 mutations. However, this finding appears insufficient to provoke the onset of leukemia [39]. Similarly, when Ts1Rh mice are mated with *Gata1*$^{\Delta e2}$ mice, the compound mice did not show more phenotypes compared to the *Gata1*$^{\Delta e2}$ mutant mice in adulthood, except for further enlargement of megakaryocyte colony sizes [43]. The Ts1Rh and *Gata1*$^{\Delta e2}$ compound mutant mice never develop leukemia. However, intriguingly, bone marrow cells are prone to transformation into leukemic cells when MPL^{W515L} are retrovirally overexpressed in the compound mutant mouse cells [43]. MPL^{W515L} is an activating mutation of the thrombopoietin receptor, which is frequently found in patients with myeloproliferative neoplasms [63]. Therefore, it seems plausible that the megakaryocyte progenitors carrying the GATA1-S mutation become sensitive to the oncogenic mutation by the presence of trisomy 21. Thus, GATA1-S mutation in combination with trisomy 21 seems to give rise to the high transformation potential, which may be involved in the increased risk of leukemogenesis in DS children.

7. Conclusion

GATA1 is a lineage restricted transcription factor that is responsible for the hematopoiesis in erythroid and megakaryocyte lineages. GATA1 dysfunction leads to two types of leukemias. One type originates from the *quantitative deficit of GATA1*, which causes accumulation of immature erythroblasts and finally triggers erythroleukemia in mice [55]. The other type originates from the *qualitative defect of GATA1* (TMD and DS-associated AMKL) [64]. In the latter case, the GATA1-S mutation is provoked in high frequency, and the mutation contributes to the pathogenesis of TMD and DS-associated AMKL [49]. This structural change causes the accumulation of immature megakaryoblasts and finally triggers megakaryoblastic leukemia in mice and humans. Mice carrying a single GATA1-S mutation develop a phenotype resembling TAM [61]. In addition, trisomy 21 alters the function of hematopoietic cells [25-27], and in cooperation with GATA1-S, it leads to the pathogenesis of TMD and DS-associated AMKL. We surmise that the mouse models discussed in this chapter will provide important insights into the pathogenesis of TMD and DS-associated AMKL.

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