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Invited Review

GATA Transcription Factors and Hematological Diseases

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HARIGAE, H. *GATA Transcription Factors and Hematological Diseases*. Tohoku J. Exp. Med., 2006, **210** (1), 1-9 —— The development of mature blood cells from hematopoietic stem cells is regulated by transcription factors that control and coordinate the expression of lineage-specific genes. The GATA family consists of six transcription factors that function in hematopoietic and endodermal development. Among them, GATA-1 is expressed in erythroid, megakaryocytic, eosinophil and mast cell lineages, and GATA-2 is expressed in stem and progenitor cells, at more immature stage compared with GATA-1. Based on the characteristic phenotypes of GATA-1 and GATA-2 mutant mice, it has been suggested that mutations of these *GATA* genes in humans may result in the onset of certain clinical diseases. To date, mutations of *GATA-1* gene have been found in inherited anemia and thrombocytopenia, and Down syndrome-related acute leukemia, which exhibits megakaryocytic phenotypes and frequently occurs in patients with Down syndrome. In contrast, no mutation of *GATA-2* gene has been identified in hematological diseases; however, we found the expression level of GATA-2 is significantly decreased in CD34 positive cells in patients with aplastic anemia. Since GATA-2 functions in the proliferation of hematopoietic stem cells, the reduction of GATA-2 expression in CD34 positive cells may result in the decreased number of hematopoietic stem cells, which is the characteristic feature of aplastic anemia. Based on these lines of evidence, some types of hematological diseases may be defined as transcription factor diseases. ———— GATA-1; GATA-2; hematopoiesis; adipogenesis; hematological disease

Hematopoiesis is maintained under a proper balance between self-renewal and differentiation of hematopoietic stem cells (HSCs). When HSCs undergo the differentiation, they progressively lose their developmental potentials and acquire the specific characteristics of mature cells. Although it remains unclear how the direction to specific lineages is determined in the differentiation process, transcription factors, which control the expression of lineage-specific genes, play key roles. GATA transcription factor was first cloned as a nuclear protein which binds to GATA sequence in Locus Control Region (LCR), one of enhancer regions of globin genes (Evans and Felsenfeld 1989; Tsai et al. 1989). It was thought to be a unique erythroid-specific transcription

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factor, however, through cDNA cloning and screening, six GATA binding proteins have been found to date (Yamamoto et al. 1990; Arccei et al. 1993; Kelly et al. 1993; Tamura et al. 1993; Laveriere et al. 1994). They share a couple of common characteristics (Orkin 1992). (1) They recognize (A/T)GATA(A/G) sequence in regulatory regions and control the gene expression. (2) They have a unique protein structure, Zn finger motif, as a DNA binding domain. (3) Their expression profiles are independently restricted, suggesting that they may activate tissue-specific genes and thereby define tissue-specificity.

To date, new animal models named as knockout mice, which carry the specifically disrupted gene, have been produced by using embryonic stem (ES) cell culture system combined with homologous recombination (Cappeci 1989). This method has brought us novel insights of functions of transcription factors on the hematological development in vivo. Mice lacking GATA transcription factors have also been generated, and shown to exhibit abnormal phenotypes as expected from their expression profiles (Pevny et al. 1991; Fujiwara et al. 1996; Takahashi et al. 1997). Since their phenotypes are similar to those of some hematological diseases, the mutation or expression levels of GATA factors have been extensively examined in human hematological diseases, and it has been clarified that aberrant expression of the GATA factor is responsible for the development of hematological diseases. In this article, clinical diseases that are caused by the mutation of two GATA factors, GATA-1 and GATA-2, are reviewed.

GATA-1

GATA-1 was the first identified GATA factor that recognizes GATA sequence existing in LCR region of globin gene (Evans and Felsenfeld 1989; Tsai et al. 1989). GATA sequence has been found in the promoter and enhancer regions of erythroid-specific genes and proved to be essential for the full transcription activity (Evans et al. 1988; Wall et al. 1988; Ponka 1997). Originally GATA-1 was thought to be the master regulatory transcription factor for erythroid differentiation, however, it is also expressed in megakaryocytes, eosinophils, and mast cells (Romeo et al. 1990; Harigae et al. 1998; Hirasawa et al. 2002) (Table 1). Since GATA-1 has been shown to be essential for normal hematopoiesis, especially for erythropoiesis and megakaryopoiesis, it is easily acceptable that the mutation of *GATA-1* gene causes characteristic hematological diseases. In fact, both inherited and acquired hematological diseases due to *GATA-1* mutation have been reported (Crispino 2005). In addition, it has been speculated by the experimental and clinical observations that altered expression levels of GATA-1 cause specific hematological disorders.

GATA-1 mutations in inherited and acquired hematological disorders

First, missense mutations have been identified in several families suffered from inherited anemia and thrombocytopenia (Nichols et al. 2000; Freson et al. 2001, 2002; Mehaffey et al. 2001) (Fig. 1). Since *GATA-1* is located on X-chromosome (Table 1), the disease is inherited in X-linked fashion. All of these mutations concentrate in the N-finger of GATA-1, which is

Table 1. Features of GATA-1 and GATA-2.

	Expression profile	Chromosome location in humans	Phenotypes of genomic disruption in mice
GATA-1	Erythroid, eosinophil, mast, megakaryocytic lineages	Xp11.23	Arrested erythroid maturation and megakaryocytic development
GATA-2	Early erythroid cells, mast, megakaryocytic lineages, multipotent progenitor cells	3q21.3	Deficit in hematopoietic stem or progenitor cells

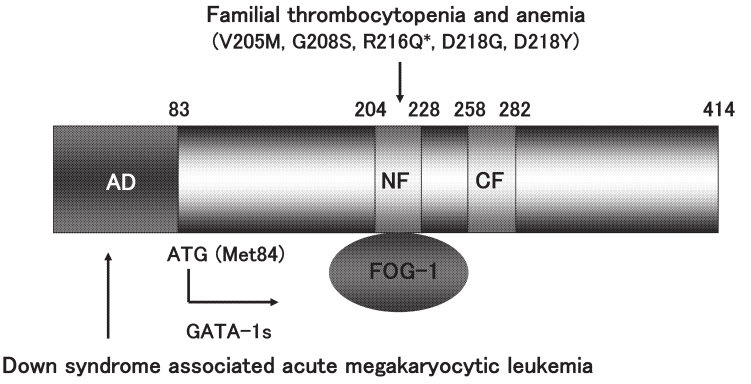

Fig. 1. Mutations of GATA-1 in human hematological diseases.
*R216Q mutation does not affect the interaction with FOG-1. AD, activation domain; NF, N-terminal zinc finger; FOG-1, friend of GATA-1; CF, C-terminal zinc finger.

important for the association of GATA-1 with its co-factor FOG-1 (friend of GATA-1); however, clinical phenotypes are different each other. Namely, V205M leads to thrombocytopenia and anemia (Nichols et al. 2000), G208S to thrombocytopenia (Mehaffey et al. 2001), D218G and D218Y to thrombocytopenia and dyserythropoiesis (Freson et al. 2001, 2002), and R216Q to thrombocytopenia with beta thalassemia (Yu et al. 2002). All mutations except for R216Q mutation, which interferes DNA binding rather than FOG-1 interaction (Yu et al. 2002), affect FOG-1 interaction. Interestingly, D218Y leads to severe anemia compared to D218G, despite the substitution at the same residue. Since D218Y leads to a profound loss of affinity of GATA-1 with FOG-1 than D218G (Freson et al. 2002), the strength of FOG-1 interaction may account for the difference of clinical phenotype.

Next, the characteristic mutations in the *GATA-1* gene have been found in an acquired hematological disease, Down syndrome (DS)-related acute leukemia (Welchsler et al. 2002; Groet et al. 2003; Hitzler et al. 2003; Mundschau et al. 2003; Xu et al. 2003; Ahmed et al. 2004). About 10% of children with DS develop the related myeloid disorder, transient myeloproliferative disorder (TMD) that is characterized by the accumulation of immature megakaryoblasts in the peripheral blood, liver, and bone marrow (Zipursky et al. 1992). The disease resolves spontaneously in most cases, however, almost 20% of the TMD cases developed acute megakaryocytic leukemia (AMKL) (Massey et al. 2006). Mutations of *GATA1* gene have been found in almost all cases of AMKL accompanied with DS.

All mutations exist in N-terminus of GATA-1 resulting in the premature stop codon, and alternative short form of GATA-1 protein (GATA-1s), which lacks the N-terminal activation domain, is translated from a downstream ATG sequence (Welchsler et al. 2002). It is obvious that this mutation is correlated with the leukemogenesis, because GATA-1s becomes undetectable in remission status after therapy (Welchsler et al. 2002). However, the mechanism by which GATA-1s induces AMKL remains unclear. Mice, in which GATA-1s is expressed from the endogenous locus, exhibit transiently an abnormal megakaryopoiesis during fetal liver hematopoiesis; however, the megakaryopoiesis and erythropoiesis in adult mice appeared to be normal (Li et al. 2005). In addition, an inherited mutation, which causes GATA-1s, has been recently reported in a family with anemia but without thrombocytopenia (Hollanda et al. 2006). These lines of evidence suggest that GATA-1s is responsible for dyshematopoiesis, but an additional genetic event(s) may be necessary for development of AMKL. The acquired GATA-1 mutation in N-terminus that induces the expression of GATA-1s has been thought to be detected only in AMKL with DS;

however, we found a same kind of *GATA-1* mutation in a patient, who did not have DS but suffered from AMKL (Harigae et al. 2004). The patient is 48-year-old female who was diagnosed as AMKL by the morphological and immunohistochemical analysis. We found insertion of 20 bp within exon 2 of the *GATA-1* gene, resulting in the introduction of a premature stop codon in the gene sequence encoding the N-terminal activation domain. This is the first case of acquired AMKL without DS, and the observations of this patient suggest that *GATA-1* mutations play an important role in the development of AMKL in non-DS adult individuals.

The *GATA-1* mutations in inherited and acquired hematological disorders are summarized in Fig. 1. Since GATA-1 is essential for erythropoiesis as well as megakaryopoiesis, it is necessary to search the mutation in erythroleukemia patients as well as AMKL patients.

Hematological disorders due to altered expression levels of GATA-1: models for idiopathic myelofibrosis and myelodysplastic syndrome

Based on the results of experiments of GATA-1 mutant mice, not only the mutations of *GATA-1* gene but also the aberrant expression of GATA-1 have been speculated to cause some types of hematological diseases. The mice whose GATA-1 expression was suppressed to 20% of normal level, referred as GATA-1(low) mice, exhibit myelofibrosis with the characteristic feature of clinical idiopathic myelofibrosis, i.e., the presence of tear-drop poikilocytes and progenitor cells in the blood, and collagen fibers in the marrow (Vannucchi et al. 2002). Consistent with this phenotype of GATA-1 (low) mice, the expression of GATA-1 protein in megakaryocytes from patients with idiopathic myelofibrosis has shown to be reduced (Vannucchi et al. 2005). These observations suggest that decreased expression of GATA-1 in megakaryocytes may contribute to pathogenesis of idiopathic myelofibrosis.

On the other hand, when GATA-1 expression is suppressed to 5% of the normal level by the interference of the promoter activity, the phenotype of mutant mice is clearly different from that of GATA-1(low) mice. The male embryos hemizygous for the knockdown mutation, whose expression of GATA-1 is reduced to 5% of normal level (*GATA-1.05*/Y), do not survive beyond 12.5 embryonic days due to severe anemia (Takahashi et al. 1998). In contrast, female mice heterozygous for the mutation (*GATA-1.05*/X) can survive, but show various degrees of anemia and thrombocytopenia. There are two types of hematopoietic cells in *GATA-1.05*/X female; one is those in which the wild-type *GATA-1* allele is activated and another is those in which mutant *GATA-1.05* allele is activated according to the random inactivation of the X chromosome. Hematopoietic cells with the activated wild-type *GATA-1* allele are able to differentiate normally, whereas the cells with the activated *GATA-1.05* allele stop their differentiation and proliferate at immature stage, and overwhelm normal hematopoietic cells with the activated wild-type *GATA-1* allele. Supporting this phenotype in vivo, *GATA-1.05/Y* proerythroblasts have shown to be accumulated in S phase and escaped from apoptosis in in vitro study (Pan et al. 2005). Interestingly, in the early stage of life, *GATA-1.05*/X mice suffered from multilineage cytopenia with immature cells in hematological tissues (Takahashi et al. 1998), and in their late stage of life, they highly developed leukemia, because the accumulated immature hematopoietic cells are eventually transformed into leukemic cells (Shimizu et al. 2004). From the view of clinical hematology, this phenotype of *GATA-1.05*/X mice in their early stage of life is similar to myelodysplastic syndrome (MDS), which is characterized by peripheral cytopenia despite of normal or hypercellular bone marrow, and progress to acute leukemia in the late stage of the disease. Sharing with the clinical course of MDS, thus, *GATA-1.05*/X mice may be a good animal model to clarify the molecular mechanism of the development of MDS.

Based on the above idea, we screened for genes that are differentially expressed in hematopoietic tissues between wild type and *GATA-1.05*/X mice. Through this approach, we found that the expression level of YB-1 mRNA is significantly increased in spleen of *GATA-1.05*/X

mice (Yokoyama et al. 2003a). YB-1 has been shown to be a multifunctional protein functioning in the transcriptional or post-transcriptional gene expression (Grant and Deeley 1993; Ladomery and Sommerville 1994; Raj et al. 1996). YB-1 is upregulated in various neoplastic tissues including bone marrow of patients with hematological disorders including MDS (Lee et al. 2001). We further examined the expression of YB-1 mRNA in erythroblasts in MDS patients as well as normal subjects. Consistent with the findings in *GATA-1.05*/X mouse, the expression level of YB-1 mRNA in glycophorin A-positive cells was increased in all of examined MDS patients compared to normal subjects (Yokoyama et al. 2003b). These results suggest the pathological role of YB-1 for developing dyserythropoiesis, which is a common feature of MDS and *GATA-1.05*/X mouse.

GATA-2

GATA-2 was cloned as one of members of the GATA family (Yamamoto et al. 1990). GATA-2 is expressed at the stem and progenitor stage, including erythroid lineage, in hematopoiesis (Table 1). In addition, GATA-2 is expressed besides hematopoietic cells, for example, in endothelial cells and undifferentiated ES cells (Dorfman et al. 1992). It has been interested how two GATA factors, GATA-1 and GATA-2, which are expressed redundantly in erythroid cells, share the roles on erythroid differentiation. When their expression levels along erythroid differentiation are examined, GATA-2 expression is strongest in the stem cell level and decline along the maturation process, while the expression of GATA-1 is upregulated (Leonard et al. 1993; Mouthon et al. 1993; Nagai et al. 1994; Labbaye et al. 1995). These expression profiles suggest that GATA-2 functions in the regulation of the proliferation and maturity of stem and progenitor cells (Yuasa et al. 2005). However, it can compensate for the role of GATA-1 in committed cells to erythroid lineage. Takahashi et al. (2000) showed that transgenic GATA-2 completely rescued a primitive erythropoiesis of GATA-1 deficient mouse. Furthermore, it was also reported that GATA-2 expression was markedly increased in erythroid cells deficient in GATA-1 (Weiss et al. 1994; Suwabe et al. 1998). Supporting these observations, when GATA-2 is overexpressed in a leukemic cell line committed to erythroid lineage, the expression levels of erythroid-specific genes including α-, β-, γ-globin and transferrin receptor were increased (Harigae et al. 2006). In addition, the binding of GATA-2 protein to the GATA element in α-globin LCR was increased in GATA-2-overexpressing cells (Harigae et al. 2006). These findings suggest that temporal and spatial regulation may be important for displaying specific functions of GATA-2.

Aberrant expression of GATA-2 in aplastic anemia

Since the expression of GATA-2 is strongest in stem and progenitor level, GATA-2 (–/–) embryos failed to survive beyond the stage of primitive hematopoiesis (Tsai et al. 1995). When chimeric mice produced with GATA-2 (–/–) ES cells were examined, GATA-2 (–/–) ES cells did not contribute to any hematopoietic organs (bone marrow, spleen, and thymus). Colony assays using bone marrow or spleen cells of these chimeras showed that erythroid, myeloid, and mixed colonies derived from GATA-2 (–/–) ES cells were not detected (Tsai et al. 1995). Since GATA-2 (–/–) mice die in utero, the function of GATA-2 in adult hematopoiesis has been examined in GATA-2 hetero knockout GATA-2 (+/–) mice. Through the analysis of GATA-2 (+/–) mice, it has been found that GATA-2 haploinsufficiency leads to the reduced number of HSCs and the increased percentage of apoptotic cells (Rodrigues et al. 2005). Furthermore, hematopoietc stem cells in GATA-2 (+/–) mice are less competitive than those in wild type when transplanted to irradiated mice, suggesting GATA-2 haploinsufficiency also leads to qualitative defects of HSCs (Ling et al. 2004).

To date, the hematological disorders associated with *GATA-2* mutation have not been identified. It has been reported that GATA-2 can interact with leukemic chimera proteins (Tsuzuki et al. 2000; Tsuzuki and Enver 2002); however, it has

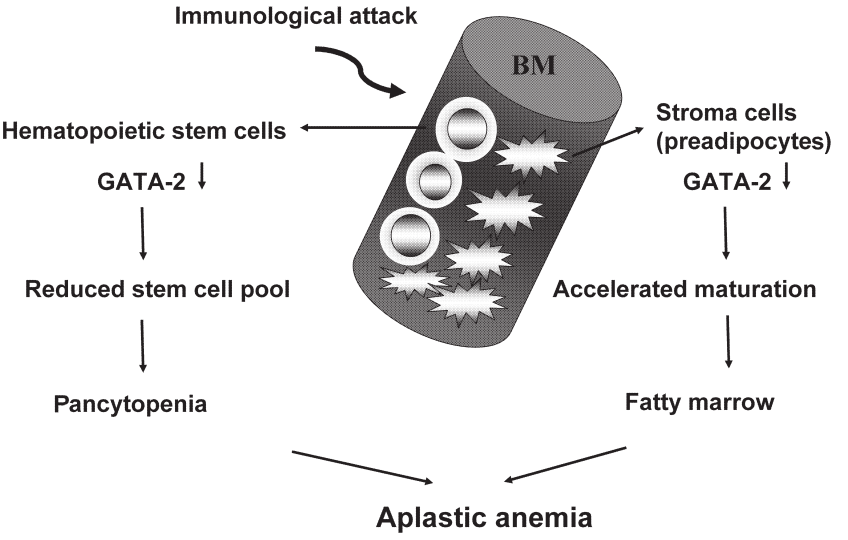

Fig. 2. Aplastic anemia and aberrant expression of GATA-2.
Down-regulation of GATA-2 in hematopoietic tissue including hematopoietic stem cells and stromal cells may result in decrease of blood cells and increase of mature adipocytes, which are characteristic feature of aplastic anemia.

not been proved that GATA-2 directly functions in leukemogenesis. From the results of in vivo and in vitro experiments, it is possible that aberrant expression of GATA-2 impairs hematopoiesis at the early progenitor level. We thus hypothesized that the reduced expression of GATA-2 results in the bone marrow failure syndrome due to impaired stem cell function such as aplastic anemia (AA). AA is characterized by a hypocellular bone marrow, reduced hematopoiesis and peripheral pancytopenia (Young 1999). The number of HSCs is reduced by a certain immunological attack in most of AA patients. In order to explore our hypothesis, we examined the levels of expression of mRNA encoding GATA-2, SCL and AML1 in CD34-positive HSCs in patients with aplastic anemia. There was a remarkable decrease of GATA-2 mRNA expression in patients with AA compared to control (Fujimaki et al. 2001). Recently, Zeng et al. (2004) performed Gene chip analysis using CD34 positive cells from AA and normal subjects, and also showed that the expression level of GATA-2 mRNA was decreased in AA. These findings indicate that there may be downregulation of GATA-2 in HSCs in AA, which may cause the reduction of number of HSCs.

To determine whether the decrease of GATA-2 expression is due to polymorphism of the *GATA-2* gene, we have examined the sequence of enhancer and promoter region that have been shown to be important for GATA-2 expression (Pan et al. 2000; Kobayashi-Osaki et al. 2005) in AA patients, however, no mutation has been detected so far. Since the number of patients is limited, no definite conclusion is drawn; however, it is possible that down regulation of GATA-2 in AA may be induced by secondary mechanism such as immunological injury. On the other hand, GATA-2 has been shown to be essential for the maintenance of immaturity of preadipocytes (Tong et al. 2000; Tsai et al. 2005). Preadipocytes are major component of stroma cells, which form hematopoietic microenviroment and support hematopoiesis. If the pathological immune response in AA influences the level of GATA-2 expression in not only HSCs but also stromal preadipocytes in the bone marrow, it may accelerate the maturation of preadipocytes, leading to the formation of fatty bone marrow, which is one of characteristics of AA. Thus, aberrant expression of GATA-2 may be a major cause for the develop-

ment of clinical features of AA (Fig. 2).

Conclusion

By the characteristic phenotypes of mutant mice, GATA factors have been proved to be indispensable for hematopoiesis. In addition, their phenotypes have prompted us to speculate that certain clinical hematological disorders may be caused by dysfunction of GATA transcription factors. The findings of basic research, together with extensive analysis of clinical samples, could contribute to clarify the pathogenesis of these hematological diseases and develop the targeting therapies.

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