主論文の要旨

GATA2 and secondary mutations in familial myelodysplastic syndromes and pediatric myeloid malignancies

（家族性骨髄異形成症候群・小児骨髄系造血器腫瘍における*GATA2*遺伝子変異および二次性遺伝子変異の解析）

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【Background】

GATA2, a member of the GATA transcription factor family, plays critical roles in hematopoiesis and vascular and neural development. Mutations in this gene have been identified as the cause of several hematologic disorders. *GATA2*-related disorders include familial myelodysplastic syndromes (MDS)/acute myeloid leukemia (AML); chronic myeloid leukemia (CML); monocytopenia and mycobacterial infection (MonoMAC) syndrome; and dendritic cell, monocyte, B and NK lymphoid (DCML) deficiency. Patients with MonoMAC syndrome or DCML deficiency exhibit increased susceptibility to infection and often progress to MDS and AML. Because *GATA2* is associated with the development of vascular and lymphatic systems, patients with *GATA2* deficiency may present with lymphedema, monosomy 7, and MDS, known as Emberger syndrome.

【Objects and Methods】

We aimed to clarify the prevalence of *GATA2* and other mutations linked to myeloid malignancies in pediatric hematologic disorders related to MDS and AML. We investigated the incidence of *GATA2* mutations in Japanese children with AML (N = 75), JMML (N = 96), AA (N = 75), and familial MDS (N = 4). All exons and intron 5 (wherein pathogenic mutations were documented) of *GATA2* were analyzed using Sanger sequencing. We performed target gene sequencing of 82 hematological malignancy-related genes in patients with GATA2 mutations.

【Results】

We detected two mutations in 75 children with AML (c.953C>T, p.A318V and c.599insG, p.S201X). In familial MDS, *GATA2* mutations were detected in all three families. We did not detect any mutations in our cohort of children with AA or JMML (**Table 1**).

We constructed three pedigrees of familial MDS (**Table 2**). The probands of Family 1 (Patient 1 and 2) were diagnosed with MDS and MonoMAC syndrome with mixed karyotypes. The younger brother (Patient 2) was treated using hematopoietic stem cell transplantation (HSCT). The proband of Family 2 (Patient 4) who suffered from Emberger syndrome died despite treatment using HSCT. Family 3 was also associated with Emberger syndrome. The mother (Patient 6) was diagnosed with lymphedema. The proband (Patient 5) was diagnosed with MDS and received HSCT.

Pedigrees constructed for three families indicated an autosomal dominant mode of inheritance for the prevalent diseases (**Figure 1A**). The probands and father from Family 1 had the same germline mutations in *GATA2* (Patient 1, 2, and 3; c.892dupT, p.C298LfsX86, **Figure 1B**). Using the history of Family 4, we inferred that

the proband had familial MDS with a confirmed germline nonsense mutation (Patient 4, c.802G>T, p.G268X). We examined all five members of Family 3 for mutations and found co-segregation of the clinical phenotype and *GATA2* mutations (Patient 5 and 6, c.1018-2A>G, splice site). All of the three germline mutations resulted in truncated proteins, wherein two zinc finger domains were lacking, suggesting a complete loss of protein function. In addition to the inherited mutation, we detected a 3-bp deletion mutation in the younger brother from Family 1 (Patient 2; c.1168_1170delAAG, p.K390del). We also confirmed these mutations in exons 4 and 6 occurred in different alleles and concluded that the c.1168_1170delAAG mutation was of somatic origin.

To further investigate somatic mutational events, we performed target gene sequencing of hematological malignancy-related genes in 6 familial MDS patients and 2 AML patients. By covering the entire coding region of 82 genes with 400x coverage, we detected 62 alterations. Based on literature and database searches, we identified 27 of these as driver mutations. Deep sequencing enabled us to measure the variant allele frequency (VAF) of each somatic mutation (**Figure 2**). In Patients 1 and 6, no somatic events were detected. In the other 4 patients, at least 2 somatic driver mutations were detected in each patient in addition to a *GATA2* germline mutation.

【Discussion】

In our study, we identified germline *GATA2* alterations in all 3 families with familial MDS and somatic *GATA2* alterations in pediatric sporadic AML (2/75, 3%). In a comprehensive study of adult AML, *GATA2* mutations were found in 2% of patients and were considered possible driver mutations. The frequency of *GATA2* mutations in our pediatric cohort was comparable with that of adult AML. Our findings support the role of *GATA2* mutations as driver mutations in AML.

Although the same germline mutation was detected in the familial members, the clinical manifestation resulting from germline mutations differed among family members. These findings corroborate previous reports in demonstrating a clear association between *GATA2* mutations and familial MDS. Our data strongly suggests that additional somatic events are modifiers of these *GATA2*-related disorders. We detected somatic driver mutations in 4 of 6 patients with familial MDS. In Family 1, Patient 2 carried two driver mutations and required HSCT. In contrast, Patient 1 carried no detectable mutations and HSCT was not required. In Family 3, Patient 6 carried no detectable somatic mutations and presented with lymphedema alone, whereas Patient 5 carried two driver mutations and required HSCT. Overall, serial clonal evolution explains at least part of the variation seen on the clinical presentation of *GATA2*-related disorders.

We detected at least 20 driver mutations in familial MDS patients. Of these, 7

involved *GATA2* (6 germline and 1 somatic) mutations. In adult MDS, somatic mutations in several genes predict clinical outcome. Therefore, it may be possible to predict the clinical course of patients with *GATA2*-related disorders by somatic mutations.

【Conclusions】

The proportion of *GATA2* mutations in children with sporadic AML, AA, and JMML was previously believed to be low. We detected the presence of not only germline mutations, but also a somatic mutation of *GATA2* in familial MDS. Newly detected secondary mutations may affect the clinical course of familial MDS and therefore, be of prognostic value for clinical decision making such as transplantation indication assessment. Further genomic investigations including whole-genome sequencing will lead to increased knowledge on secondary mutations in *GATA2*-related disorders.