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GATA-1 and *GATA-2* gene expression is related to the severity of dysplasia in myelodysplastic syndrome

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TO THE EDITOR

Dysplasia of erythroid, granulocytic and megakaryocytic lineages is the diagnostic hallmark of myelodysplastic syndrome (MDS). The usual result is one or more peripheral blood cytopenias and an increased risk of leukemic transformation. Transcription factors play a key role in controlling the cellular proliferation and differentiation of hematopoietic stem cells.[1,2] GATA-1 and GATA-2 are zinc-finger transcription factors that play an important role in gene regulation during development and differentiation of hematopoietic cells. Previous studies using *GATA-1* and *GATA-2* knock-out mice showed that *GATA-2* gene inactivation caused partial deficiency in the production of hematopoietic lineages through its action in early progenitor or stem cell proliferation,[2] while *GATA-1* was important for erythroid differentiation of hematopoietic progenitor cells.[3] The expression of GATA family genes and its implication in primary MDS has never been reported. Since inappropriate hematopoietic differentiation results in varying degree of myelodysplasia, which is the hallmark of MDS, we hypothesized that mRNA expression patterns of *GATA-1* and *GATA-2* are associated with MDS, and possibly with the severity of these diseases.

In this study, bone marrow (BM) specimens were obtained from 45 adult patients with primary MDS, including 24 patients with refractory anemia with excess of blasts or refractory anemia with excess of blasts in transformation (RAEB/RAEBT) and 21 patients with refractory anemia or refractory anemia with ring sideroblasts (RA/RARS). Peripheral blood (PB) samples from three normal individuals and BM specimens from eight patients with non-hematological diseases were used as controls. Two independent hematologists examined the BM aspirate specimens and trephine biopsy sections. Morphological features that were used to indicate dyserythropoiesis, dysgranulopoiesis and dysmegakaryopoiesis were based on the FAB group[4] (Table 1). A scoring system based on features listed in Table 1 was developed to split the MDS patients into two groups morphologically according to the severity of myelodysplasia: severe or minimal dysplasia. The presence of less than one-third of the features was considered to be minimal dysplasia. This scoring system has been shown to correlate well with the degree of peripheral cytopenias in MDS; in addition, patients with severe dysplasia are more likely to be transfusion dependent (unpublished observations).

GATA-1 and *GATA-2* gene expression was determined by using Superscript one-step RT-PCR system (Gibco BRL, Gaithersburg, MD, USA).[5] The primers used in this study were human *GATA-1* (forward 5′-TGCTCTGGTGTCCTCCACAC-3′ [121–140]; reverse 5′-TGGGAGAGGAATAGGCTGCT-3′ [604–623]), human *GATA-2* (forward 5′-AGCGTCTCCAGCCTCATCTTCCGCG-3′ [745–769]; reverse 5′-CGAGTCTTGCTGCGCCTGCTT-3′ [1016–1035]) and human β-actin as control (forward 5′-CCGGCTTCGCGGGCGACG-3′; reverse 5′-TCCCGGCCAGCCAGGTCC-3′).[6–10] Briefly, One-step RT-PCR was performed in 50 μl reaction containing 1 μg total RNA, 10 pmol of each sense and antisense primers, 200 U SuperScript II H– reverse transcriptase, 2.5 U Platinum Taq DNA polymerase, 1×RT buffer, 0.2 mM of dNTP and 1.2 mM of $MgSO_4$.

We detected *GATA-1* in only five of the 45 MDS patients (11.1%) and *GATA-2* in 40 patients (88.9%) (Figure 1). Since *GATA-1* expression may be auto-regulated or controlled by other *GATA* family members including *GATA-2*,[11,12] we attempted to divide the MDS patients into one of the following four groups according to the expression of *GATA-1* and *GATA-2* genes; *GATA-1*$^+$*GATA-2*$^+$, *GATA-1*$^-$*GATA-2*$^+$, *GATA-1*$^-$*GATA-2*$^-$ and *GATA*-1$^+$*GATA-2*$^-$. We identified 35 (77.8%) MDS patients with a *GATA-1*$^-$*GATA-2*$^+$ expression pattern, five patients (10.9%) were either *GATA-1*$^+$*GATA-2*$^+$ or *GATA-1*$^-$*GATA-2*$^-$ (Table 2). The expression of *GATA-1* and *GATA-2* was not detected in any samples obtained from hematologically normal control subjects; in addition, the results obtained from MDS samples were identical irrespective of the source of cells (BM or PB). These indicate that the cells used were free of contaminating erythroblasts, thus GATA expression is in the lymphoid population and the cells isolated from individuals with normal hematopoiesis did not express these genes. Therefore, the current results are likely to represent characteristics of MDS cells rather than contaminating erythroid or progenitor cells.

Thirty-three (73.3%) MDS patients exhibited severe dysplasia of erythroid, granulocytic and megakaryocytic lineages, while the remaining 12 patients had minimal dysplasia. Most patients (28 of 33; 84.8%) who showed severe dysplasia were GATA-1$^-$GATA-2$^+$, and the remaining five patients were GATA-1$^-$GATA-2$^-$. In contrast, all patients with *GATA-1* and *GATA-2* coexpression had minimal dysplasia. We did not observe any correlation between the pattern of GATA expression and FAB subtype (Table 2) or karyotype (data not shown). These results, although based on a small number of patients, suggest that dysregulation of GATA gene expression is a feature of myelodysplastic hematopoiesis and that the pattern of expression is linked to the degree of dysplasia ($P < 0.05$).

The coordination of several transcription factors is essential in the development of normal hematopoiesis.[13,14] The observations derived from *GATA-1* and *GATA-2* gene knockouts support a hypothesis that these transcription factors act successively and co-operatively with overlapping and unique functions during normal erythropoieisis. In stem cells or early hematopoietic progenitors, *GATA-2* expression predominates to promote cellular proliferation. Rising levels of *GATA-1* suppress *GATA-2* expression by an unknown mechanism and sustain *GATA-1* expression through positive autoregulation. The shift from *GATA-2* to *GATA-1* predominance tips the cellular balance in favor

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Table 1 Major features of dysplasia in myelodysplastic syndrome

Cell line	*Peripheral blood*	*Bone marrow*
Erythroid	Macrocytes Anisopoikilocytosis Polychromatic cells Punctate basophilia Pappenheimer bodies	Bi- or multi-nuclearity Nuclear lobulation and fragmentation Howell–Jolly bodies Megaloblastoid features Cytoplasmic vacuolation
Granulocytic	Pelger anomaly Hypogranularity or agranular Increased nuclear projection Gross hypersegmentation	Irregular bodies of coalesced granules Neutrophil changes as in the peripheral blood Left shift or increased blast Promyelocytes with absent or sparse granules Hypogranularity or agranularity of myelocytes Chromatin clumping
Monocytes	Multiple elongated lobes Fine azurophilic granules	As in peripheral blood
Megakaryocytic	Giant platelets Agranular platelets Megakaryocyte fragments	Micromegakaryocytes Binuclear/hypolobulated Irregular nucleus Polynuclear forms

The presence of less than one-third of the listed features is considered to be minimal dysplasia.

Table 2 Severity of dysplasia and expression of GATA-1 and GATA-2

Study group	*No. of patients*			
	Severe dysplasia	*Minimal dysplasia*	*RAEB/RAEB-T*	*RA/RARS*
GATA-1$^-$GATA-2$^+$	28 (94.8%)	7 (15.2%)	20	15
GATA-1$^+$GATA-2$^+$	0	5 (100%)	4	1
GATA-1$^-$GATA-2$^-$	5 (100%)	0	0	5
Total No. of patients	33	12	24	21

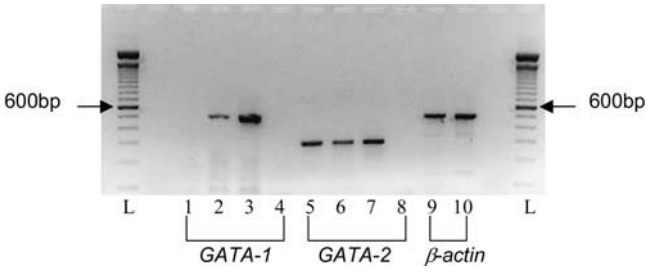

Figure 1 Representative of the *GATA-1, GATA-2* gene and *β-actin* PCR products in primary MDS and controls (lanes 2, 3, 5–7) by RT-PCR. L = 100 bp DNA ladder (GIBCO, BRL). Lanes 1–4 using primer *GATA-1* (L1 = normal person; L2 and L3 patients with primary MDS; lane 4 = negative control (without RNA)); lanes 5–8 using primer *GATA-2* (L5–7 = patients with primary MDS; lane 8 = negative control). Amplification of *β-actin* as a control for the presence of intact RNA is shown in lanes 9 (normal person) and 10 (MDS patient). Sizes are in base pairs (bp).

of terminal differentiation. We detected *GATA-1* transcripts in only 10% of the MDS patients; in addition, all *GATA-1*-negative patients showed severe dysplasia. In contrast, most of our MDS patients expressed *GATA-2*. The overexpression of *GATA-2* in the absence of *GATA-1* suggests that *GATA-1* negatively regulates *GATA-2*. The loss of *GATA-1* expression in the majority of our MDS patients suggests that *GATA-1* is essential for differentiation of all the hematopoietic lineages. Previous studies had shown that terminal erythroid maturation might require downregulation of *GATA-2*.[15] Consistent with these observations, either absence or persistence of *GATA-2* in combination with downregulation of *GATA-1* in our patients could explain the dysregulation of cellular differentiation in MDS, and *GATA-1* negativity is strongly associated with severity of dysplasia. However, among the 12 patients with minimal dysplasia, seven patients were found to be *GATA-1*$^-$*GATA-2*$^+$. In the absence of *GATA-1*, there may be other factors, eg *GATA-3* gene, that promote differentiation and maturation of hematopoietic cells resulting in minimal dysplasia.

Our findings suggest a critical role for *GATA-1* and *GATA-2* in the development of MDS. Inappropriate expression of these transcription factors may lead to impaired hematopoietic differentiation, which is reflected in the dysplastic nature of hematopoietic cells that is characteristic of MDS. Further studies are required to examine the prognostic value of GATA transcription factors in determining the outcome of patients with primary MDS.

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The GSTM1 and GSTT1 genetic polymorphisms and susceptibility to acute lymphoblastic leukemia in children from north Portugal

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TO THE EDITOR

Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer accounting for approximately 25–30% of all childhood malignancies.[1,2] The origin of this disease can be explained by a combination of genetic and environmental factors, and therefore, like many other cancers, it is considered a complex disease, caused by the 'carcinogenetic' effect of the environment modified by a series of genes. Many of these genes tend to occur in allelic forms representing functional polymorphisms partially explaining inter-individual variability in cancer susceptibility.[1] However, chemical carcinogens are not reactive *per se*: they require metabolic activation before interacting with genetic material that may lead to mutations and eventually the initiation of cancer. The glutathione S-transferase mu-1 (GSTM1) and glutathione S-transferase theta-1 (GSTT1) are phase II enzymes that have the ability to detoxify numerous electrophilic compounds including the activated carcinogens.[1] Both GSTM1 and GSTT1 exhibit genetic polymorphism in populations with a large percentage of individuals displaying a homozygous deletion of the structural genes. Moreover, an increased frequency of GSTT1 and GSTM1 null genotypes (either in individual or combined status) has been associated with several malignancies.

Particularly concerning ALL, the studies until now performed besides being very scarce, have reported contradictory results for associations between GSTM1 and GSTT1 and cancer predisposition. For instance, Krajinovic *et al*[3] analyzing a case-control study that involved a white population of Canadians with French origin, reported a significant association between the presence of the GSTM1 null genotype and an increased risk of ALL. For GSTT1, the authors have not detected any apparent role in the etiology of ALL. However, in a previous study, Chen *et al*[2] have found that only the double null genotype for GSTM1 and GSTT1 was significantly more frequent among a sample of black children with ALL from USA, although failing to show a similar association among the white children analyzed. The discrepancies can be partially explained as the result of interpopulational differences in genetic backgrounds of susceptibility and/or of geographical differences in environmental exposure to carcinogens.[2,3] Therefore, extensive population-specific studies are needed.

For studying in north Portugal the relationship between GSTM1 and GSTT1 genotypes and risk of susceptibility to ALL, we have applied the most recently described GSTM1 and GSTT1 genotyping procedures, which allow the unambiguous detection of the three genotypes that can result from deleted and non-deleted alleles.[4,5] The detection of the deletions was PCR-based using the primers described by Kerb *et al*[4] and Sprenger *et al*[5] For both GSTM1 and GSTT1, the null alleles were co-amplified with the normal ones in a single reaction tube, and thereafter the amplified products were electrophoresed. For GSTT1, a 1460 bp fragment corresponded to the null allele and a fragment of 540 bp to the functional one (Figure 1). For GSTM1, fragments of 8073 bp or 4748 bp indicated the presence of a functional allele or of a deleted allele, respectively (Figure 2).

Patients enrolled in the study were 47 children with ALL, in various clinical phases, all undergoing the same chemotherapy protocol. As controls, 102 healthy subjects not following any therapeutic treatment and born in the same geographic area as patients, north Portugal, were analyzed.

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