J Hematopathol (2015) 8:169–176
DOI 10.1007/s12308-014-0225-0

REVIEW ARTICLE

Myeloid proliferations associated with Down syndrome

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Received: 27 August 2014 / Accepted: 19 November 2014 / Published online: 14 December 2014

Abstract A subset of Down syndrome (DS) (trisomy 21) neonates is born with a unique erythromegakaryocytic myeloproliferative disorder that spontaneously resolves over the first few months of life (DS-transient abnormal myelopoiesis (DS-TAM); previously called DS-transient myeloproliferative disorder (DS-TMD) and DS-transient leukemia (DS-TL)). These infants are at high risk for developing subsequent acute megakaryoblastic leukemia (myeloid leukemia associated with Down syndrome (ML-DS); previously called DS-acute megakaryoblastic leukemia (DS-AMKL)). The molecular basis for DS-TAM/ML-DS remained mysterious for a long period of time. However, new genetic insights have been gained over the past 12 years that have begun to decipher the pathophysiology of this unusual disorder.

Keywords Down syndrome · Myeloid proliferation · Myeloproliferative disorder · *GATA1*

Introduction

A subset of Down syndrome (DS) (trisomy 21) neonates is born with a unique erythromegakaryocytic myeloproliferative disorder that spontaneously resolves over the first few months of life (DS-transient abnormal myelopoiesis (DS-TAM); previously called DS-transient myeloproliferative disorder (DS-TMD) and DS-transient leukemia (DS-TL)). These infants are at high risk for developing subsequent acute megakaryoblastic leukemia (myeloid leukemia associated with Down syndrome (ML-DS); previously called DS-acute megakaryoblastic leukemia (DS-AMKL)). The molecular basis for DS-TAM/ML-DS remained mysterious for a long period of time. However, new genetic insights have been gained over the past 12 years that have begun to decipher the pathophysiology of this unusual disorder.

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Epidemiology

The true incidence of DS-TAM has been difficult to discern since not all DS infants receive blood count analysis in the neonatal period and it is likely that many asymptomatic cases go unnoticed. In addition, clear diagnostic criteria for DS-TAM have not been established [1]. The World Health Organization (WHO) defines DS-TAM as "increased" peripheral blasts in neonates with DS but does not provide cutoff values for "increased" blasts [1, 2]. This is particularly problematic since nearly all DS neonates have blasts present in their peripheral blood [1]. Earlier retrospective studies estimated that ~5–10 % of neonates with DS have clinically apparent DS-TAM [3, 4]. Pine et al. [5] performed PCR amplification and *GATA1* gene Sanger sequencing from dried blood spots of 585 neonates with DS. They detected *GATA1* mutations (a recently identified molecular hallmark of DS-TAM (see "Genetics" below)) in 22 (3.8 %) of the samples. They also noted a small increased incidence of *GATA1* mutations for Hispanic compared to non-Hispanic babies. A recent prospective population-based study in the UK collected clinical data, peripheral blood counts, peripheral blood morphology, and *GATA1* mutational analysis on 200 DS neonates [1]. Using the criteria of peripheral blood blasts of >10 % (by morphology) and a *GATA1* mutation detected by Sanger

sequencing/denaturing high-performance liquid chromatograph (DHPLC) analysis followed by next-generation sequencing, the authors found an incidence of 8.5 %. However, they also detected 18 cases of "silent" DS-TAM in which *GATA1* mutations were detected by next-generation sequencing, but no hematologic abnormalities compared to non-*GATA1*-mutated DS neonates were evident. If one includes these silent DS-TAM cases, then the incidence in this study would be 17.5 %. Thus, the true incidence of DS-TAM depends on how it is defined and what methods are used to detect *GATA1* mutations, but it is likely to be in the 4–18 % range. Additional large prospective population-based studies, as well as adoption of strict universal diagnostic criteria and sequencing methods, will be required to better define the true incidence of DS-TAM and whether it differs among ethnic groups.

About 20–25 % of patients with "clinically apparent" DS-TAM that survive the neonatal period subsequently develop ML-DS [6–9]. The mean age for presentation of ML-DS is about 20 months (range, ~6–38 months) [6, 7]. ML-DS can also develop from silent DS-TAM [1]. Overall, children with DS have ~500-fold increased risk of developing AMKL compared to the general population [4].

Clinical features

The clinical presentation of DS-TAM is highly variable. The majority of patients are asymptomatic. They are typically diagnosed incidentally after blood counts are obtained for other reasons and reveal abnormalities consistent with DS-TAM. A subset of infants presents with more severe manifestations, which can include fetal hydrops, liver failure, jaundice, coagulation defects, bleeding diasthesis, heart failure, pleural effusions, ascites, and/or respiratory failure. Symptomatic patients present either as stillborns or within the first 3 weeks of life [4]. Hepatomegaly is common. There is frequent megakaryocytic liver infiltration and hepatic fibrosis. Peripheral blood analysis reveals thrombocytopenia or thrombocytosis, elevated white blood cell with excess blasts, and frequently nucleated red blood cells. The full clinical picture may not be apparent until the second or third week of life. Remarkably, the peripheral blasts and other DS-TAM symptoms typically self-resolve by ~3–4 months of age (~36–49 days from diagnosis) [6–9].

Trisomy 21 is typically constitutional in DS-TAM/AMKL. However, a substantial proportion of patients can have trisomy 21 mosaicism or harbor germ line translocations involving chromosome 21 [7]. Therefore, the absence of typical DS physical features does not exclude the diagnosis of DS-TAM.

Subsequent development of ML-DS is frequently preceded by a myelodysplastic syndrome (MDS)-like phase that can last for months [10]. This is characterized by worsening thrombocytopenia followed by anemia, ineffective erythropoiesis, and dysplastic changes of megakaryocytic and erythroid precursor cells on bone marrow exam.

Morphology

The peripheral blood smear of DS-TAM shows significant polychromasia, abundant nucleated red blood cells, and the presence of blasts (Fig. 1). Large-sized platelets are usually present and sometimes megakaryocyte fragments can be seen. The blasts in ML-DS exhibit typical French–American–British (FAB) M7 morphology [6]. They have a scant deeply basophilic cytoplasm with cytoplasmic blebbing, open chromatin, and prominent nucleoli (Figs. 2 and 3). Bi-nucleated erythroblasts are sometimes observed on bone marrow exam (Fig. 3).

Immunochemistry and expression profiling

The blasts in DS-TAM and ML-DS are positive for CD7, CD33, CD34, CD36, CD38, CD41, C42b, CD45, CD61, CD71, CD117, glycophorin A, TPO receptor (TPO-R), and IL-3 receptor alpha (IL-3Rα) [6, 7, 11] (Table 1). They are negative for CD10, the EPO receptor (EPO-R), and IL-6 receptor alpha (IL-6Rα), and variably positive for CD4 (Dim), CD13, and CD56.

Histochemical staining of DS-TAM blasts is negative for periodic acid–Schiff (PAS) and typically negative or weakly staining for myeloperoxidase and Sudan Black [6] (Table 2). Staining for acid phosphatase and esterase is usually positive.

ML-DS samples have distinct gene expression profiles compared to non-DS-AMKL samples [12]. Many of the key differences are not explained by the simple presence of an extra copy of chromosome 21 genes. These include over expression of *c-Kit*, *c-MYC*, and *GATA2*, known direct GATA1-repressed target genes involved in early progenitor cell survival and proliferation. These findings along with the unique clinical features of ML-DS versus non-DS-AMKL suggest that the two leukemias represent distinct molecular entities.

Genetics

GATA1 mutations in DS-TAM and ML-DS

A major advance in the understanding of the molecular basis of DS-TAM and ML-DS came in 2002 when Wechsler and colleagues [13] identified somatic mutations in the gene encoding the key megakaryocytic/erythroid transcription factor GATA1 in all cases of ML-DS examined. This was rapidly

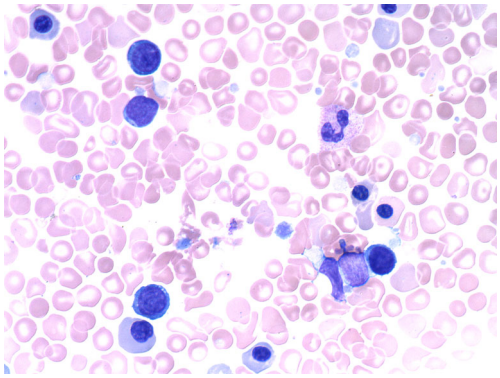

Fig. 1 Wright–Giemsa-stained peripheral blood smear of a patient with DS-TAM. The blasts have a scant basophilic cytoplasm. Note the presence of significant polychromasia, abundant nucleated red blood cells, and large-sized platelets

followed by a number of reports identifying similar mutations in DS-TAM as well as in additional cases of ML-DS [14–18]. With rare exceptions, these types of *GATA1* mutations have never been reported in non-DS individuals. The *GATA1* mutations are not detectable in remission samples from patients treated successfully for ML-DS, indicating their close linkage to the disease [13, 17]. It is now recognized that *GATA1* mutations occur in all cases of DS-TAM and ML-DS and that it should be considered a molecular hallmark of the disorder [1].

A wide variety of *GATA1* mutations have been described, including missense, splice-site, deletions, insertions, and duplications, but all target exon 2 (or rarely exon 3) [19]. All of the mutations lead to introduction of a premature stop codon. However, in these cases, translation initiates from an in-frame ATG located at codon 84. This produces a short GATA1 isoform (~40 kDa), called "GATA1s" that lacks an N-terminal transactivation domain (Fig. 4). Low levels of GATA1s are produced normally during development but decrease relative to full-length GATA1 during terminal erythroid differentiation [20, 21]. In contrast to wild-type cells, GATA1s

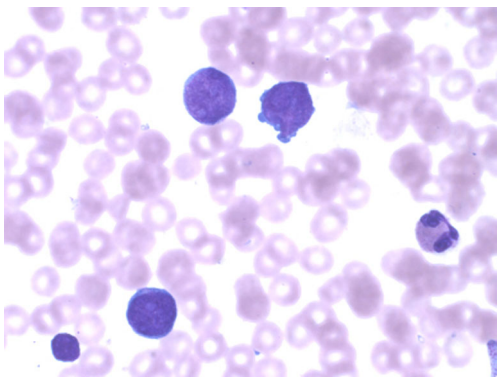

Fig. 2 Wright–Giemsa-stained bone marrow aspirate of a patient with ML-DS. ML-DS blasts are shown, some with characteristic cytoplasmic blebbing

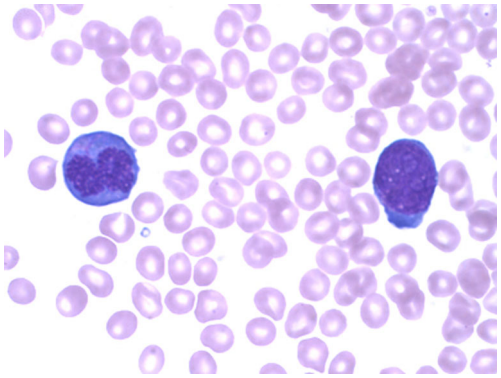

Fig. 3 Wright–Giemsa-stained bone marrow aspirate of a patient with ML-DS showing a bi-nucleated erythromegakaryocyte precursor cell

is produced exclusively in the mutant cells (the *GATA1* gene is located on the X-chromosome).

GATA1 plays essential roles in terminal erythroid [22] and megakaryocyte maturation [23], and contributes to eosinophil [24] and mast cell development [25, 26]. GATA1 deficient murine megakaryocytes markedly hyperproliferate in liquid culture and fail to complete their full maturation [27]. Mice containing megakaryocyte-selective GATA1 deficiency are thrombocytopenic, have impaired megakaryocyte maturation, and develop bone marrow fibrosis as they age [23, 28]. Remarkably, knock-in mice that exclusively express GATA1s have developmental stage-specific effects on megakaryocyte proliferative control [29]. Yolk sac and early fetal liver derived megakaryocytes markedly hyperproliferate. However, late fetal liver and adult bone marrow megakaryocytes proliferate close to normal. These findings indicate that developmental stage-specific factors modulate the effect of GATA1s on megakaryocyte proliferative control and may be involved in the spontaneous remission of DS-TAM. The mechanism(s) underlying these stage-specific effects of GATA1s remain incompletely understood. Involvement of the insulin-like growth factor (IGF) and mTOR signaling pathways [30] and/or type I interferon signaling pathways may be involved [31].

Studies of dried blood spots from neonates who developed DS-TAM or ML-DS [32] as well as prospective studies of infants with DS-TAM/ML-DS [1, 33] have identified the simultaneous presence of multiple distinct *GATA1* mutant

Table 1 Immunophenotypic surface markers for DS-TAM/ML-DS blasts (based on [6, 7, 11])

Signal	Marker
Positive	CD7, CD33, CD34, CD36, CD38, CD41, C42b, CD45, CD61, CD71, CD117, glycophorin A, TPO receptor, IL-3 receptor alpha
Variable/dim	CD4 (Dim), CD13, CD56
Negative	CD10, EPO receptor, IL-6 receptor alpha

Table 2 Histochemical phenotype of DS-TAM blasts (based on [6])

Signal	Stain
Usually positive	Acid phosphatase
	Esterase
Variable	Sudan Black
	Myeloperoxidase
Negative	Periodic acid–Schiff (PAS)

clones. With rare exceptions, GATA1s-generating mutations have not been detected in large numbers of healthy non-DS newborns or non-DS-AMKL. Collectively, these findings suggest that a trisomy 21 genetic background creates a strong selective pressure for clones containing GATA1s-generating mutations during in utero development. However, the molecular basis for this remains unknown.

GATA1s-generating mutations have been detected as early as 21 weeks of gestation [34]. In the cases that have been analyzed, *GATA1* nucleotide changes in the ML-DS cells are identical to those found in the preceding DS-TAM cells (or a subclone) from the same patient [1, 17, 33]. These findings support a clonal evolution model, in which the GATA1s mutations are early in utero initiating events that predispose to later development of ML-DS [14, 16] (Fig. 5).

The role of trisomy 21

GATA1s-generating mutations by themselves appear to be insufficient to cause a transient myeloproliferative disorder or megakaryoblastic leukemia in humans. This is based on the observation of a family containing a germ line GATA1 exon 2 (GATA1s-generating) mutation in the absence of trisomy 21 [35]. Male members from several generations have macrocytic anemia and neutropenia, but normal platelet counts. None of the affected individuals have had clinically evident neonatal transient myeloproliferative disorder or have developed leukemia.

A number of recent studies have documented that trisomy 21, in the absence of *GATA1* mutations, leads to perturbed fetal and postnatal hematopoiesis [36–39]. This includes significantly increased number of hematopoietic stem cells (HSCs) and megakaryocyte–erythroid progenitor (MEPs) in the trisomy fetuses compared to chromosome 21 disomy fetuses. Moreover, these cells have significantly enhanced cell-autonomous clonogenic and proliferative potential. There is also impairment of B-cell development. In vitro differentiation of human-induced pluripotent stem cells (iPS) from trisomy 21 individuals largely recapitulates these hematologic abnormalities compared to isogenic disomy 21 control cells [40]. A mouse model of DS, the Ts65Dn mouse, develops a highly penetrant myeloproliferative disorder with significant thrombocytosis, megakaryocyte hyperplasia, megakaryocyte dysmorphology, and bone marrow fibrosis, in the absence of *GATA1* mutations [41]. Thus, it seems likely that the underlying genetic background in trisomy 21 contributes to the molecular pathogenesis of DS-TAM and ML-DS beyond the initial selection for GATA1s-generating mutant clones.

A number of candidate genetic factors on chromosome 21 have been investigated for their role in DS-TAM/AMKL. Supporting data has been reported for *ERG* [42, 43], *ETS2* [44], and the microRNA miR-125b [45]. Interestingly, *RUNX1*, which is also located on chromosome 21 and encodes a key megakaryocytic transcription factor that physically associates with GATA1 and is frequently mutated in human leukemia, does not appear to be involved [12, 41].

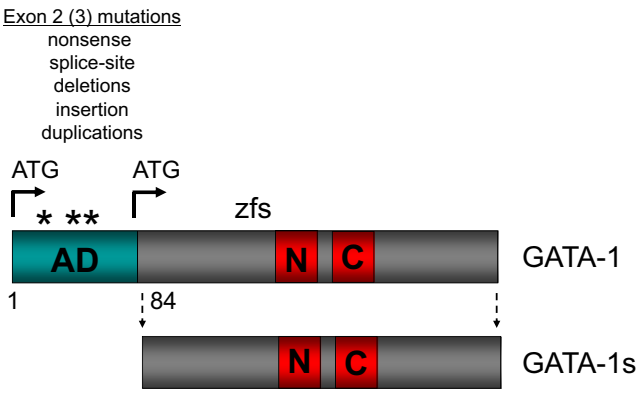

Fig. 4 Schematic drawing of protein product produced by DS-TAM/ML-DS-related GATA1 mutations. Full-length protein is shown in the *top panel* with the two carboxyl terminal zinc finger (*zf*) domains indicated. *TA* transcriptional activation domain. *Asterisks* represent mutations in exon 2 (or exon 3) that introduce premature stop codons. The alternate translational start site at codon 84 is indicated, and the protein product ("*GATA1s*") generated from translation from this site is shown below. (Adapted from [13, 19])

Secondary mutations leading to ML-DS

While the combination of GATA1s mutations and trisomy 21 is likely sufficient to produce DS-TAM, they appear to be insufficient to generate ML-DS. Current data suggests that the acquisition of additional genetic perturbations in a preexisting DS-TAM (GATA1 mutant containing) clone is required for development of full blown ML-DS. Acquired cytogenetic aberrations and copy number alterations are common in ML-DS and include trisomy 8, loss of chromosome 5/7 material, gain of chromosome 21, dup(1q), del(16q), and other rarer abnormalities [46, 47]. Somatic point mutations in *JAK1* [48], *JAK2* [48], *JAK3* [48–50], *TP53* [51], *FLT3*, and *MPL* have been described in small subsets of cases. A recent study of 41 DS-TAM, 49-ML-DS, and 19 non-DS-AMKL cases using genomic profiling, whole exome, and/or whole genome sequencing identified a high proportion of cases with somatic mutations in genes encoding components of the cohesin

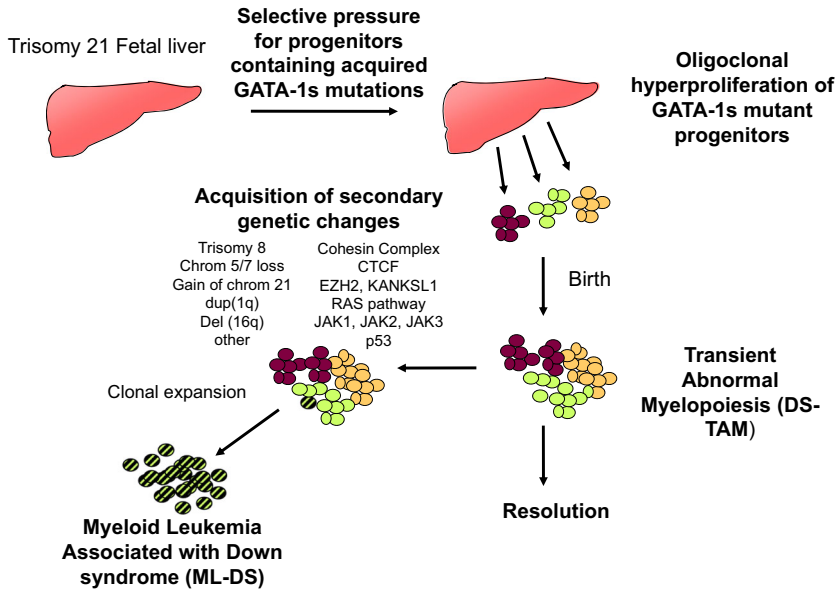

Fig. 5 Clonal evolution model of ML-DS. Mutant GATA1s progenitor cell clones emerge during early fetal hematopoiesis under strong selective pressure in a trisomy 21 genetic background. Multiple clones may emerge. Megakaryocyte–erythroid progenitor cells from these clones markedly hyperproliferate under the influence of GATA1s. These clones are either extinguished or reverse their hyperproliferative phenotype during the transition to postnatal hematopoiesis, reflecting the spontaneous remission of DS-TAM. In some cases, additional genetic events occur in a subclone(s) that develops into ML-DS. (Adapted from [61])

complex (53 %) or the cohesin-binding insulator factor CTCF (20 %) in ML-DS [33]. Mutations in these genes were not found in any of the DS-TAM samples and only 11 % of non-DS-AMKL. Additional somatic mutations in genes encoding epigenetic regulators (including *EZH2* and *KANKSL1*) and signaling pathway factors (including RAS pathway genes and *SH2B3* (*LNK*)) were also seen in the ML-DS samples. The molecular significance of these findings remains unknown at this time but strongly suggests that additional genetic events, particularly in the cohesin complex, collaborate with GATA1s and trisomy 21 to generate ML-DS.

Prognosis

Most children (~75–90 %) spontaneously clear their peripheral blasts and normalize their blood counts over the first few months of life [6]. However, despite the transient nature of DS-TAM and its mild presentation in many patients, ~11–20 % of clinically apparent DS-TAM cases result in perinatal death, usually due to fulminant liver failure, disseminated intravascular coagulation, and/or multiple effusions [6–8, 52]. Treatment with low dose cytarabine (Ara-C; 0.5 to 1.5 mg/kg/day for 3 to 12 days) is effective in severe neonatal presentations [7].

For infants that develop ML-DS, the overall prognosis is considerably better than patients with non-DS-AMKL. Treatments with current chemotherapy protocols (which include Ara-C) produce long-term event-free survival rates of around 80 % [47, 53–55]. Up-front bone marrow transplantation is typically not indicated given the excellent cure rates with conventional chemotherapy. The reason for the strong response to therapy in ML-DS versus non-ML-DS is not known. It has been proposed that low levels the cytarabine metabolizing enzyme cytidine deaminase, whose gene is transcriptionally activated by GATA1, in ML-DS may contribute [56]. Despite the excellent response to therapy, toxic deaths remain a problem and occur in ~7 % of treated cases [47].

Potential predictive factors

During the neonatal DS-TAM phase of the disease, risk of early death is associated with high white blood cell count (WBC) ($>100\times10^9$/L), ascites, preterm delivery, bleeding diasthesis, direct hyperbilirubinemia, increased liver enzymes, and failure to clear peripheral blasts [6–8]. For ML-DS, a normal karyotype (except trisomy 21) is associated with increased incidence of relapse (~21 %) compared to cases with aberrant karyotypes (~9 %) [47]. Other independent predictors of poor event-free survival in ML-DS include WBC $\geq20\times10^9$/L and age >3–4 years at diagnosis [47, 55].

Summary and perspectives

In summary, DS-TAM and ML-DS are characterized at the molecular pathophysiologic level by the constellation of GATA1s-producing mutations, trisomy 21, fetal origin, and

acquisition of additional genetic events in the progression to ML-DS. Fortunately, with the exception of certain cases of severe neonatal presentations, the overall prognosis and cure rate for infants with DS-TAM/ML-DS is excellent. Additional investigation will be necessary to: (1) fully understand the strong selective pressure for GATA1s mutant containing clones during fetal hematopoiesis in trisomy 21 individuals; (2) identify the specific genetic component(s) on chromosome 21 that contributes to the disorder; (3) fully uncover the mechanisms underlying the spontaneous remission of DS-TAM; and (4) elucidate the key genetic steps that lead to progression to ML-DS.

Clinical efforts are underway to develop less intensive therapies in order to reduce the toxic death rates and morbidity associated with ML-DS treatment. Important clinical questions yet to be addressed include (1) whether mutational screening for GATA1s mutations in all DS neonates is clinically useful; (2) whether quantitative measurement of GATA1s mutant clone levels is clinically useful in following patients with known GATA1s clones; and, (3) if tumor bulk reduction during the DS-TAM phase of the disease (such as with low-dose AraC) would decrease the frequency and/or severity of subsequent ML-DS development. Additional recent and detailed reviews on DS-TAM/AMKL can be found in ref. [4, 57–60].

Acknowledgments This work was supported by the National Institutes of Health grant P01 HL32262.

Conflict of interest The author declares no conflict of interest.

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