Chapter 14
Down Syndrome-Associated Hematologic Disorders and Leukemia

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Transient Abnormal Myelopoiesis

Introduction

Transient abnormal myelopoiesis (TAM), also referred to as transient myeloproliferative disorder (TMD) or transient leukemia (TL), is characterized by increased circulating myeloid blasts that have an acquired GATA-binding protein 1 (*GATA1*) mutation in children with Down syndrome [2].

Epidemiology

TAM occurs in around 20–30% of children with Down syndrome. Approximately 10–15% of neonates with Down syndrome have blasts >10%, and clinical and hematological features of TAM, referred to as clinical TAM, whereas another

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10–15% have acquired *GATA1* mutations with a low number of blasts (<10%) and no clinical or hematological features suggestive of TAM, the so-called silent TAM [2, 3]. Clinical TAM most commonly manifests during the early neonatal period. The median age at presentation is 3–7 days; most cases will manifest by 2 months but can be diagnosed up to 6 months old [4, 5]. TAM has been reported in partial trisomy 21 and trisomy 21 mosaicism [6, 7].

Clinical Features

Clinical features of TAM vary; typical presenting features include hepatomegaly, splenomegaly, pericardial/pleural effusions, and skin rash [2, 3, 8]. Down syndrome children who have silent TAM may be diagnosed on review of peripheral blood smear and complete blood count (CBC) but otherwise appear well clinically; on the other hand, patients may be very sick with disseminated leukemic infiltration with massive hepatosplenomegaly, effusions, coagulopathy, and multi-organ failure [8]. Characteristic hematologic features of TAM include leukocytosis, anemia, and thrombocytopenia. Leukocytosis in TAM shows increased circulating blasts, neutrophilia, monocytosis, basophilia, and increased myelocytes. Other laboratory features are abnormal liver function tests and abnormal coagulation [2, 3, 8].

Morphology and Immunophenotyping

Many cases of TAM are morphologically and immunophenotypically indistinguishable from acute megakaryoblastic leukemia. Blasts in the peripheral blood and bone marrow show typical features of megakaryoblasts, which include a modest amount of agranular basophilic cytoplasm with cytoplasmic blebs, irregular nuclear contours, and a nuclear chromatin pattern that may be more condensed than traditional myeloblasts with infrequent nucleoli (Fig. 14.1). The blast percentage in the peripheral blood may be disproportionately higher than in the bone marrow, reflecting probable megakaryopoiesis in the liver [4, 9]. If a bone marrow biopsy is performed, multilineage dysplasia may be seen, most often with involvement of megakaryocytic and erythroid cell lines. Cases with prominent dysplasia may more closely resemble a conventional myelodysplastic syndrome. Marrow fibrosis is variable. Peripheral basophilia may be present. Liver biopsies performed for liver dysfunction may show necrosis and/or fibrosis, which is usually accompanied by atypical myeloid progenitors, including megakaryoblasts, within sinusoids. Examination of the placenta may show marked expansion of the chorionic plate and stem vessels by the circulating myeloid cells (Fig. 14.2) and/or features of fetal thrombotic vasculopathy.

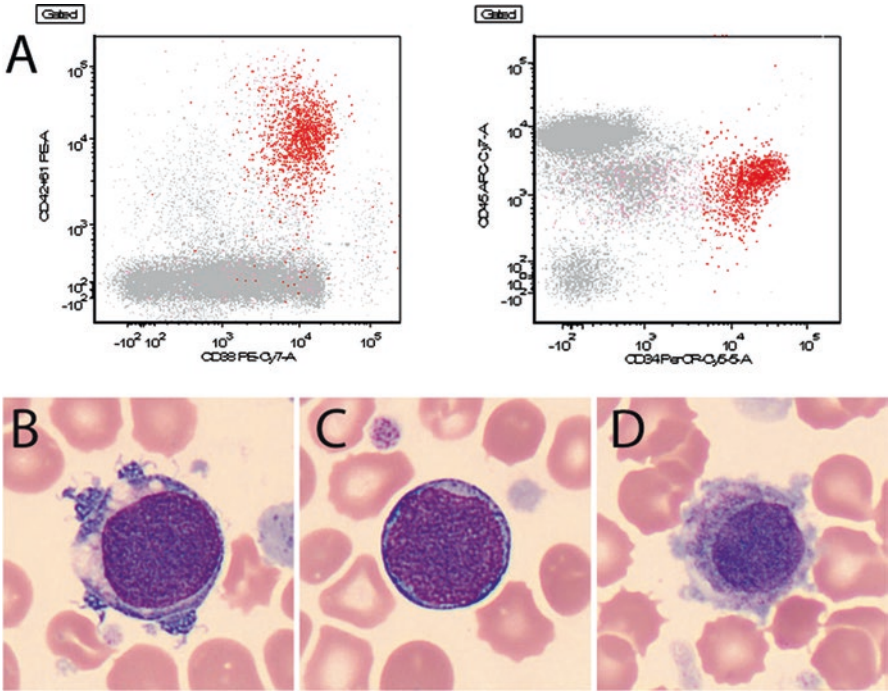

Fig. 14.1 Peripheral blood findings in TAM in a 2-week old. (**a**) Flow cytometry *dot plots* show a 5% population of cells expressing megakaryocytic markers CD42 + CD61 corresponding to megakaryoblasts; the blasts are also positive for CD34, CD117, CD45 (dim), CD13, CD33, CD11b, CD38, and CD71. (**b**–**d**) Peripheral blood smears show leukocytosis with circulating megakaryoblasts (**b**–**c**, Wright-Giemsa 1000×) and other abnormal megakaryocytic precursors (**d**, Wright-Giemsa 1000×). Cytoplasmic pseudopod formation is a characteristic feature of megakaryoblastic differentiation (**b**), but a more constant feature is the presence of deeply basophilic agranular cytoplasm with features otherwise typical for myeloblasts (**c**)

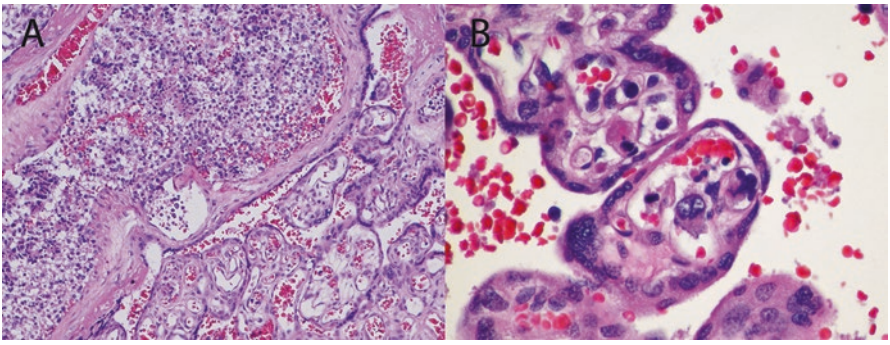

Fig. 14.2 Placenta findings in TAM. Stem vessels are expanded by an atypical myeloid infiltrate (**a**), and atypical megakaryocytic precursors are visualized within the fetal vasculature of chorionic villi (**b**) (case provided by Edwina Popek, DO, Baylor Collge of Medicine/Texas Children's Hospital, Pavilion for Women)

Immunophenotyping of Blasts by Flow Cytometry

In the majority of cases, blasts are positive for CD34, CD56, CD117, CD13, CD33, CD7, CD4 dim, CD41, CD42, CD36, CD61, CD71, TPO-R, and IL-3R [5]. Myeloperoxidase is negative. HLA-DR expression is variable. Overall, the immunophenotype of blasts is variable and there is no reliable morphologic or immunophenotypic profile to allow distinguishing between blasts from patients with TAM (with *GATA1* mutation) and those without *GATA1* mutation [2, 3]. Subtle immunophenotypic differences between TAM and Down syndrome-associated acute myeloid leukemia have been described; CD34, CD56, and CD41 are less frequently expressed in Down syndrome-associated acute myeloid leukemia (see discussion below) [5].

Cytogenetics and Molecular Findings

Clonal cytogenetic abnormalities are generally absent in TAM. There are isolated reports of oligoclonal abnormalities preceding progression to Down syndrome-associated acute myeloid leukemia (DS-ML) [10]. Detection of karyotypic abnormalities in TAM may indicate clonal evolution with progression to DS-ML [11].

The molecular events involved in the development from TAM to DS-ML have been recently reviewed [2]. Briefly, it is best described as a three-step model in fetal liver-derived hematopoietic stem or progenitor cells involving (1) trisomy 21, (2) an acquired *GATA1* mutation, and (3) at least one additional oncogenic mutation (Fig. 14.3). Of note, step 3 will be discussed in the DS-ML section below.

Constitutional trisomy 21, the first in the three-step model, leads to altered myeloid progenitor self-renewal, altered lineage development, and increased clonogenicity of megakaryocyte precursors (Fig. 14.3) [12–14]. Trisomy 21 leads to increased megakaryocyte-erythroid progenitors (MEP) and increased size and characteristics of immunophenotypic characteristics of the hematopoietic stem cell (HSC) compartment; HSC and multipotent myeloid progenitors in trisomy 21 fetal liver proliferate more and have increased erythroid-megakaryocyte output and gene expression (increased HSC with MK/E bias) [12]. Although megakaryocytes (MK) are increased, their differentiation is impaired, leading to thrombocytopenia in both the fetal and neonatal periods, suggesting that dysmegakaryopoiesis occurs in Trisomy 21 [2, 12]. Increased expression of various genes on chromosome 21, particularly *ERG* and *DYRK1a*, may be responsible for the abnormal megakaryopoiesis seen in Down syndrome [2]. On a larger scale, trisomy 21 causes genome-wide alterations in gene expression, directly or indirectly affecting multiple genes on most chromosomes [2, 15].

Acquired somatic mutations in GATA-binding protein 1 (*GATA1*), the second of the previously mentioned three-step model, serve as the "second hit" in the development of abnormal myeloid proliferations and are a defining feature of both TAM

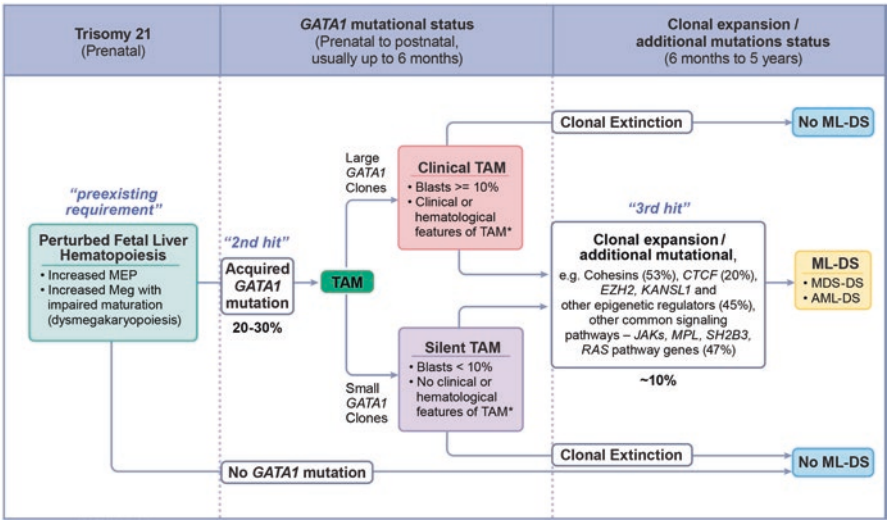

Fig. 14.3 Natural history with stepwise progression of TAM to DS-ML. The pathogenesis begins in utero with perturbed fetal liver hematopoiesis due to constitutional trisomy 21. Acquired *GATA1* mutations occur in a subset of infants with trisomy 21, either prenatally or shortly after birth, and serve as the "second hit" leading to either clinical or silent TAM. The "third hit" comes with the accumulation of additional genetic events leading to clonal expansion and promotion of leukemogenesis (Adapted from Fig. 1 of Ref. [2])

and DS-ML (Fig. 14.3). It was previously thought that *GATA1* aberrations occur in 4–10% of neonates with Down syndrome [16–18]; however, with the relatively recent discovery of silent TAM, acquired *GATA1* mutations are present at higher frequency, occurring in 25–30% of all neonates with Down syndrome and preceding all cases of DS-ML [2, 3]. The affected site of acquired *GATA1* mutations is in exon 2, or less commonly exon 3, or at the intronic boundary of exons 1 and 2. Insertions, deletions, and duplications, overall accounting for three-quarters of all mutations, are more common than point mutations. The *GATA1* gene, located at Xp11.23, encodes a zinc finger transcription factor required for normal megakaryopoiesis and erythropoiesis. The mutated gene encodes a truncated protein devoid of its amino terminus that is thought to impair *GATA1*-mediated regulation of other transcription factors such as *GATA2*, *MYB*, *MYC,* and IKAROS family zinc finger 1 (*IKZF1*); this in turn is thought to block megakaryocyte differentiation [7].

The unique combination of constitutional trisomy 21 and *GATA1* mutations is responsible for the development of TAM [7]. In fact, *GATA1* mutations in the absence of trisomy 21 cause anemia and neutropenia without leukemogenesis [19]. Additional transforming events (the third step of the three-step model), however, are required for progression to acute leukemia (discussed in section on DS-ML) (Fig. 14.3). Aside from *GATA1*, *JAK3* mutations have uncommonly been reported (1 in 15 cases in one series) [20].

Prognosis and Therapy

Spontaneous remission occurs in the majority of cases, usually within the first 3–6 months of life. The overall disease resolution rate is 85–90%, with a mortality rate of up to 20% [8, 21, 22]. Notable complications as a result of hyperviscosity and/or megakaryocyte-derived cytokines include liver fibrosis/liver failure, heart and respiratory failure, and disseminated intravascular coagulation. Low-dose cytarabine may be administered in such cases. High-risk features include failure of spontaneous remission, markedly elevated white blood cell count greater than 100 × 10^9/L, hepatic or renal dysfunction, hepatic fibrosis, prematurity, coagulopathy, massive organomegaly causing respiratory compromise, and hydrops [12, 21]. Progression to myeloid leukemia occurs in 20–30% of cases and usually occurs between 2 and 4 years of age.

Molecular markers are not currently utilized to monitor for disease progression to DS-ML. Transcriptional differences between TAM and DS-ML have been demonstrated in a single study that may have diagnostic utility. *CDKN2C* and *PRAME* transcripts were shown to be increased in DS-ML, while *MYCN* transcripts were increased in TAM [23]. An immunohistochemical study targeting the PRAME protein failed to discriminate between cases of TAM and DS-ML [24]. For the time being, the distinction between TAM and DS-ML relies predominantly on the integration of clinical parameters, hematologic features, pathologic, cytogenetic, and molecular findings (Table 14.1).

Conclusion

TAM (both clinical and silent TAM) occurs in up to a third of infants with Down syndrome. *GATA1* alterations play a key role in the pathogenesis. Spontaneous remission is seen in the majority of cases, but subsequent development of DS-ML may occur. Children with TAM require close follow-up to monitor for a possible development of DS-ML in a subset of these patients. Consensus clinical guidelines with regard to the detection and monitoring of acquired *GATA1* mutation by molecular methods have not yet been established.

Myeloid Leukemia Associated with Down Syndrome

Introduction

There is a 150-to-500-fold increased risk of developing acute myeloid leukemia (AML) in children with Down syndrome compared to the general population, with an overall incidence of 0.5–2% in this population [16, 25]. Approximately 70% of

Table 14.1 Clinical, pathologic, and molecular features of TAM vs. DS-ML

Parameter	TAM	DS-ML
Clinical		
Age	3–7 days, up to 6 months	1–2 years, up to 4 years
Organomegaly, organ dysfunction, etc.[a]	Variable	Variable
Hematologic		
Leukocytosis	Variable	Variable
Blast percentage	Variable, often high	Variable
Anemia	Usually present	Usually present
Thrombocytopenia	Usually present	Usually present
Morphology		
Blasts with megakaryoblastic differentiation	Present	Present
Erythroid and megakaryocytic dysplasia	Usually present	Usually present
Reticulin fibrosis	Variable	Usually present
Immunophenotype	Coexpression of CD34, CD56, CD41, CD61, and CD36 is common, along with other myeloid markers. Aberrant CD4 and CD7 expression may be seen	Similar to TAM, with less frequent expression of CD34, CD56 and CD41
Karyotypic abnormalities	Generally absent	+8, +11, +21, −7, dup(1q), del(6q), del(7p), dup(7q), del(16q), der(3q), low hyperdiploidy, pseudodiploidy
Molecular aberrations[b]		
Acquired GATA1 mutation	Present	Present
Additional driver genetic events	Generally absent or not sufficient for leukemogenesis	Generally required for leukemogenesis

PB peripheral blood, *BM* bone marrow
[a]Clinical manifestations may include but are not limited to hepatomegaly, splenomegaly, effusions, skin rash, coagulopathy, multi-organ failure
[b]Refer to Fig. 14.3

AML in Down syndrome manifests as acute megakaryoblastic leukemia. Myeloid leukemia associated with Down syndrome (DS-ML) is recognized as a distinct entity by the 2016 revision of the World Health Organization classification based on its distinct clinical and molecular features in comparison to other types of AML [26]. As a note on terminology, older texts separate Down syndrome-associated AML from Down syndrome-associated myelodysplastic syndrome (MDS) because some children with Down syndrome present with cytopenias, dyspoiesis, and fewer

than 20% blasts, thus meeting the criteria for MDS. By the current WHO classification schema, the term "myeloid leukemia associated with Down syndrome" encompasses both AML and MDS since they represent a continuum of the same biologic process, and all cases fulfilling the criteria for MDS will inevitably evolve into AML [5, 26].

Epidemiology

DS-ML develops 1–3 years following TAM, with a mean age of 1–1.8 years at diagnosis, but can occur up to 4 years old. Previously, it was thought that DS-ML may develop without prior TAM [26, 27]; however, recent data suggested all DS-ML preceded by TAM, either clinically or silently, the so-called clinical TAM or silent TAM, respectively (refer to the TAM section, above). It is therefore most likely that patients who develop DS-ML without a prior history of TAM are those who have silent TAM which was not diagnosed earlier. Previous studies based on retrospective data suggest that 20–30% of neonates with TAM (in general imply clinical TAM) will subsequently develop DS-ML. In consideration of the recent discovery of silent TAM, which suggests a much higher frequency of *GATA1* mutations at birth, and the estimated population-based frequency of DS-ML, which is approximately 0.5–2% before 4 years of age, the risk of progression is probably closer to 5–10% [2, 7, 16, 28]. Progression to DS-ML may immediately follow clinical TAM but more commonly occurs following a period of clinical remission.

Clinical Features

DS-ML is most often characterized by an indolent clinical course with initial myelodysplasia and progressive pancytopenia, particularly thrombocytopenia and leukopenia, with low circulating blast counts for many months followed by the eventual development of overt AML [2].

Morphology and Immunophenotyping

Morphology in the peripheral blood and bone marrow may be indistinguishable from TAM (Fig. 14.4). Blasts have megakaryoblastic features. Erythroblastic differentiation may also occur but is rare (Fig. 14.5). Megakaryocytic/erythroid dysplasia is often present. Erythroid precursors may show megaloblastic change in addition to dysplastic forms. Dysgranulopoiesis may also be present. Reticulin

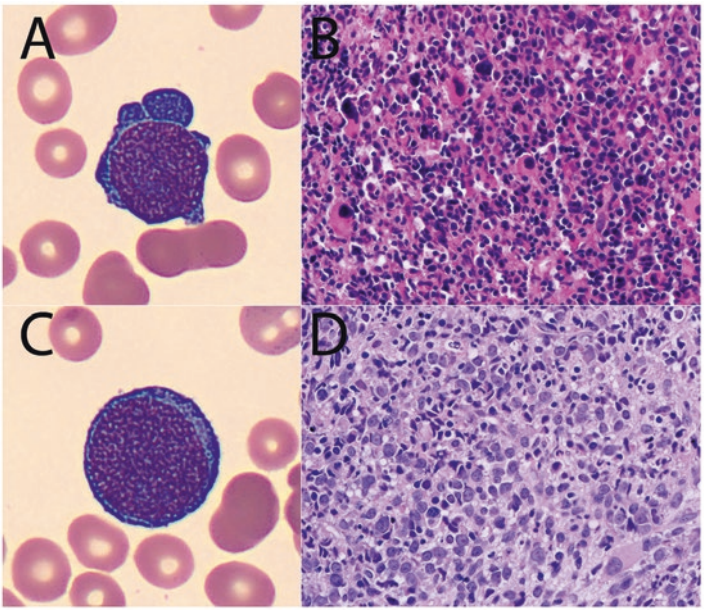

Fig. 14.4 Bone marrow findings in myeloid leukemia associated with Down syndrome, with features resembling myelodysplastic syndrome (**a**, **b**) and features of acute megakaryoblastic leukemia (**c**, **d**). Cases behaving as myelodysplastic syndrome show uni- or multilineage dyspoiesis including megakaryocytic dysplasia in a background of reticulin fibrosis. (**a**) Blasts show features of megakaryoblastic differentiation with deep basophilic cytoplasm and cytoplasmic blebs. In panel **b**, megakaryocytes are increased in number and show abnormal nuclear forms including small, hypolobated forms and nuclear hyperchromasia. In this case, there is no increase in blasts (**a** Wright-Giemsa 1000×, B H&E 500×). Cases behaving as acute megakaryoblastic leukemia show more than 20% marrow involvement by leukemic blasts showing megakaryoblastic differentiation. (**d**) Bone marrow core biopsy shows large aggregates of blasts, in a background of dyspoiesis with reticulin fibrosis (**c** Wright-Giemsa 1000×, D H&E 500×)

fibrosis in the marrow is variable but often present; therefore, it is essential to obtain a bone marrow trephine core biopsy for a more accurate assessment of blast involvement.

Immunophenotype

The usual immunophenotype is similar to TAM. In the majority of cases, blasts show megakaryoblastic differentiation and are positive for CD117, CD13, CD33, CD7, CD4 dim, CD42, CD36, CD61, CD71, TPO-R, and IL-3R. Myeloperoxidase is negative. HLA-DR expression is variable. In contrast to TAM, CD34 is negative in 50% of cases, and CD56 and CD41 are negative in 30% [5].

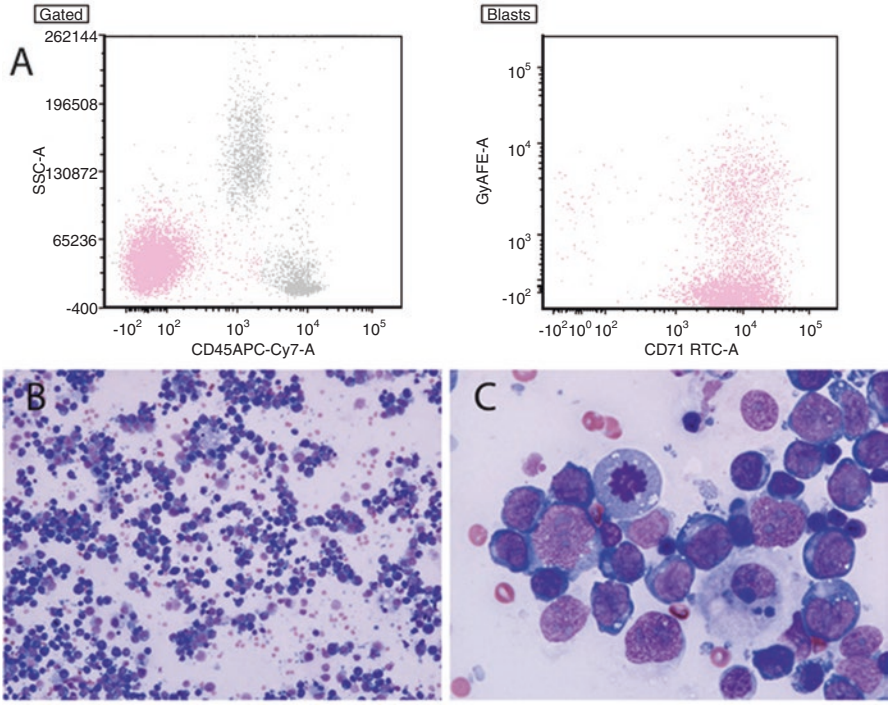

Fig. 14.5 Pure erythroid leukemia arising in a 2-year-old boy with Down syndrome. (**a**) Flow cytometry dot plots showing 62% blasts which are CD45 negative with co-expression of CD71. Glycophorin A is positive in a small subset. The blasts also express CD34 (subset), CD117, CD7 (partial), CD99, and CD33 (variable). (**b**, **c**) Aspirate smears show numerous erythroblasts with deeply basophilic cytoplasm and round nuclei with fine chromatin and one or more nucleoli. Cytoplasmic vacuolization is present (**b** Wright-Giemsa 200×, **c** Wright-Giemsa 1000×)

Cytogenetics and Molecular Findings

Karyotypic abnormalities may indicate clonal evolution of TAM to DS-ML [11]. Cytogenetic changes in DS-ML appear to be distinct from those observed in other subtypes of AML. Trisomy 8 commonly occurs in 13–44% of cases [28, 29]. Other less common abnormalities include dup(1q), del(6q), del(7p), dup(7q), del(16q), der(3q), +8, +11, +21, and −7 [21, 27, 30]. Interestingly, an additional nonconstitutional chromosome 21 is more frequent in DS-ML than in conventional AML. The recurrent cytogenetic abnormalities in conventional AML such as t(8;21), 11q23 translocations, and inv(16) are significantly less common in DS-ML. The most common ploidy levels are low hyperdiploidy and pseudodiploidy [30].

As previously elucidated in the previous section, patients with Down syndrome have trisomy 21 (first step) as a pre-existing event that disturbs normal hematopoiesis. *GATA1* mutations (second step), as a required pre-leukemic event, are found in all TAM. Additional molecular events (third step), however, are almost certainly required for progression from TAM to AML, the details of which have not been fully elucidated (Fig. 14.3).

A Down Syndrome Critical Region (DSCR) on chromosome 21 has been identified, which is a 4.3–5.4 Mb region located at 21q22 that contains several candidate genes involved in leukemogenesis including *ERG*, *ETS2*, *RUNX1*, *GABPA*, *BACH1,* and *DYRK1A* [7, 12]. Among these, *ERG* and *RUNX1* have received the most attention.

ERG expression is a poor prognostic indicator in adult T-ALL and cytogenetically normal AML, but its role in childhood leukemia is uncertain [31]. Overexpression has been demonstrated in non-DS acute megakaryoblastic leukemia, but it is not overexpressed in induced pleuripotent stem cell (iPS)-derived Down syndrome progenitors, an in vitro model of trisomy 21 [13, 32]. *ERG* causes immortalization of hematopoietic progenitor cells in *GATA1* mutant mice through JAK/STAT pathway activation, and a TMD-like effect has been demonstrated in mice models (Ts65Dn) [33, 34]. Furthermore, Ts65Dn mice that are converted from trisomy *ERG* to disomy show complete reversal of the myeloproliferative phenotype [35]. While its direct role in leukemogenesis is unclear, *ERG* and *DYRK1A* are important mediators of abnormal megakaryopoiesis [2].

RUNX1 plays an etiologic role in a subset of AML, but there is conflicting evidence for its role in DS-ML, especially since *RUNX1* trisomy does not appear to be required for the development of DS-ML [36]. *RUNX1* has a joint role with *GATA1* in megakaryopoiesis [37]. Increased expression has been demonstrated in acute megakaryoblastic leukemia, but it is not overexpressed in iPS-derived Down syndrome progenitors [13, 38].

Several other candidate genes have been proposed for which excellent reviews are available elsewhere [7]. MicroRNAs including miR-125b-2 on chromosome 21 and miR-486-5p on chromosome 8 have been implicated. Epigenetic targets on chromosome 21 including *BRWD1*, *HLCS*, and *HMGN1* may play a role [39, 40]. Candidate genes not located on chromosome 21 include *JAK3*, *MYCN*, *MYC*, *PRAME, CDKN2C,* and *EZH2*. *JAK3* mutations have been detected in a small subset of DS-ML [20]. *CDKN2C* and *PRAME* transcripts were shown to be increased in DS-ML, while *MYCN* transcripts are increased in TAM [23]. This latter observation seems to have the most potential thus far for distinguishing cases of DS-ML from TAM, although current diagnostic algorithms do not incorporate molecular testing.

The first comprehensive molecular landscape of DS-ML was achieved by Yoshida et al. whereby the authors demonstrated clonal evolution of TAM to DS-ML through acquisition of additional mutations. The major evolutional targets identified in this

study were as follows: cohesin components genes (i.e., *RAD21*, *STAG2*, *SMC3,* and *SMC1A*) (53%), *CTCF* (20%); *EZH2*, *KANSL1* and other epigenetic regulators (45%); and common signaling pathways, such as the *JAK* family kinases, *MPL*, *SH2B3* (*LNK*), and *RAS* pathway genes (47%) [41].

The molecular pathogenesis involved in DS-ML has yet to be worked out, but current knowledge supports *GATA1* mutation as an essential pre-leukemic event in the development of DS-ML. Additional transforming events, possibly involving genes in the DSCR region, lead to progression to acute leukemia [2, 7].

Prognosis and Therapy

Prognosis is overall good, and response to therapy is superior in DS-ML in comparison to non-DS AML, with 88% versus 42% 4-year disease-free survival [27]. Age greater than 4 years is associated with worse outcomes and is thought to represent a different cohort of patients [42]. In contrast to conventional AML, white blood cell count at diagnosis is not predictive of outcome [27].

Cytarabine and anthracyclines are key therapeutic agents in treatment protocols for DS-ML. This is based on the apparent increased sensitivity to DNA-damaging chemotherapeutic agents in this population [8, 43]. The increased sensitivity profile has been attributed to oxidative stress and altered folate metabolism in DS-ML, yielding deficient DNA repair mechanisms, a notion supported by the observed overexpression of genes involved in oxidative/folate metabolism including cystathionine beta synthase (*CBS*) and zinc-copper superoxide dismutase (*SOD1*) [43]. It has been proposed that this environment of oxidative stress and disrupted folate metabolism may in fact contribute to the development of *GATA1* mutations in the DS population [43]. Other proposed mechanisms contributing to the increased sensitivity include altered drug metabolism [7]. The observed sensitivity profile to these agents has permitted dose-reduction strategies in the DS-ML population that achieve the same cure rates with less treatment-related toxicity [43]. Targeted therapies have not yet been investigated in this population.

Conclusion

DS-ML may occur in individuals with Down syndrome, and trisomy 21 serves as a pre-existing event (step 1). *GATA1* mutations serve as an additional required pre-leukemic event (step 2). Additional molecular alterations must occur for promotion of leukemogenesis, the details of which are not yet fully characterized (step 3). The proposed three-step model (Fig. 14.3) is the best model for leukemogenesis based on the most recent available studies.

Down Syndrome-Associated Acute Lymphoblastic Leukemia

Introduction

There is a 20-fold increased risk of developing acute lymphoblastic leukemia (ALL) in children with Down syndrome compared to the general population. In contrast to DS-ML, there is not sufficient molecular or clinical evidence to support Down syndrome-associated ALL (DS-ALL) as a distinct biologic entity by the current WHO classification [5, 44]. However, recent advances including the discovery of *CRLF2* translocations in B-ALL have shed light on the molecular pathogenesis in at least a large proportion of DS-ALL.

Epidemiology

Children with Down syndrome have a 20-fold increased risk for ALL compared to children without Down syndrome [1]. In contrast to the general population where the ALL:AML ratio is 4–6.5:1, from birth to 15 years of age, ALL occurs with only minimally higher frequency than acute myeloid leukemia (ALL:AML ratio of 1.2–1.7:1) in children with Down syndrome [7].

Morphology and Immunophenotyping

Morphologic findings are identical to non-DS-ALL. Typical lymphoblasts have scant agranular cytoplasm, coarse-to-fine chromatin, and often indistinct nucleoli, but variations in morphology may be seen.

Immunophenotype

More than 90% of DS-ALL exhibit a precursor B phenotype. Precursor B cell neoplasms express CD45 (dim) and B-lineage markers CD19, CD22, CD20 (often weak or absent), CD24, CD79a, and PAX-5. TdT is expressed in the majority of cases. Expression of immunoglobulin is variable but often absent. CD10, CD133, CD34, HLA-DR, and CD99 may be seen. Myeloid antigens such as CD11b, CD13, CD15, and CD33 may be seen in DS-B-ALL. A minority of DS-ALL are of precursor T cell origin with expression profiles identical to non-DS T-ALL [5, 45].

Cytogenetics and Molecular Findings

A normal karyotype (aside from constitutional trisomy 21) is observed in 40% DS-ALL, which is a much greater proportion compared to non-DS pediatric ALL (7%). Rearrangements of *CRLF2* are reported in a large proportion of DS-ALL (discussed below). Favorable cytogenetic profiles including t(12;21)(p13;q22);*ETV6-RUNX1*, double trisomy of 4 and 8, and high hyperdiploidy are significantly less common in DS-ALL; t(12;21)(p13;q22) and hyperdiploidy were found in 10% of cases in the largest series [30]. Unfavorable cytogenetic findings, including *BCR-ABL1* fusion and *MLL* rearrangements, are also less common. The largest series to date reported three abnormalities +X, t(8;14)(q11;q32), and del(9p) as unique changes seen in DS-ALL although these findings have not been definitively corroborated as recurrent abnormalities [30]. Acquired +21 is fairly frequent in DS-ALL, although less so than non-DS-ALL.

Three predominant molecular aberrations have been described in DS-ALL: *CRLF2* overexpression, *JAK2* alterations, and *RAS* mutations. Constitutional trisomy 21 likely plays a role, a theory corroborated by data from non-DS ALL with intrachromosomal amplification of chromosome 21 (iAMP21).

CRLF2 Overexpression

Dysregulation of the cytokine receptor-like factor 2 (*CRLF2*) is found in 60% of DS-ALL compared to 10% of non-DS pediatric ALL [46–49]. *CRLF2* is located on the X and Y chromosomes, at Xp22.3 and Yp11.3. The protein product of *CRLF2* forms a heterodimer receptor for thymic stromal lymphopoietin (TSLP) in combination with interleukin-7 receptor subunit alpha (IL-7Rα) [1]. Receptor binding of TSLP results in activation of the STAT3, STAT5, and JAK2 pathways. Dysregulation of this pathway may result from translocations, deletions, or point mutations of the *CRLF2* gene. Translocations resulting in *CRLF2* rearrangement with *IGH* on chromosome 14 result in *CRLF2* gene overexpression [46, 48]. By an alternate mechanism, deletions in the pseudoautosomal region 1 (PAR1) of Xp22.3/Yp11.3 result in a *P2RY8-CRLF2* fusion, leading to *CRLF2* overexpression [47]. The latter mechanism is more frequent. Lastly, point mutations at codon 232 (F232C) result in *CRLF2* overexpression and have been documented in 9% of DS-ALL patients and 21% of adult B-ALL patients [1]. Of note, interleukin-7 receptor (IL-7R) gene mutations have been reported in B- and T-ALL in association with aberrant *CRLF2* overexpression, although such mutations have not specifically been described in DS-ALL [50]. A subset of DS-B-ALL patients who carry *CRLF2* gene translocation may be classified as "*BCR-ABL1*-like ALL," which is a provisional entity as proposed by the 2016 updated WHO classification schema [26].

JAK2 Mutations

JAK2 mutations may occur in combination with *CRLF2* fusions, conferring a likely cooperative effect [1]. JAK2 is a cytoplasmic protein-tyrosine kinase that catalyzes the transfer of the gamma-phosphate group of adenosine triphosphate to the hydroxyl groups of specific tyrosine residues in signal transduction molecules [51, 52]. The downstream effectors of JAK2 are a family of transcription factors called signal transducers and activators of transcription (STAT) proteins. JAK-STAT signaling plays an important role in B lymphopoiesis. Deletion of five amino acid residues from positions 682 to 686 within the JH2 pseudokinase domain on exon 14 of *JAK2* (JAK2ΔIREED) or other mutations in the R683 residue on exon 16 lead to activated JAK2 signaling in some B-ALLs [53, 54]. The majority of R683 mutations reported lead to replacement of arginine, a basic residue, with a neutral amino acid, which leads to altered interactions at the C-terminal kinase domain and results in constitutive JAK2 activation [54]. *JAK2* R683 abnormalities have been reported in 18–20% in DS-ALL [54–56]. Mutations in the *JAK2* kinase domain, as well as the *JAK1* pseudokinase domain, have also been reported with much less frequency [47]. Interestingly, *JAK2* V617F, mutations occurring in the pseudokinase domain at a different site compared to *JAK2* R683, are common mutations in myeloproliferative neoplasms but are not seen in DS-ALL [1].

Ras Mutations

Mutations in *NRAS* and *KRAS* have been reported to occur with a frequency similar to *JAK2* mutations and *P2RY8-CRLF2* fusions (15 of 42 cases in one study), and often occur as later events [57]. *RAS* and *JAK2* mutations are mutually exclusive events. Current data support the idea that *CRLF2* fusions serve as the initiating event in leukemogenesis, while *JAK2* or *RAS* mutations may arise later in the disease as subclones [57].

Contribution of Constitutional Trisomy 21

The contribution of constitutional trisomy 21 to the development of DS-ALL is less clear but it almost certainly plays a role. Interestingly, there is an overall reduction in committed B-lineage progenitors in the trisomy 21 mouse model (Ts1Rhr) with a relative increase in less mature forms. These trisomic pro-B-cells display the capability for increased self-renewal [58]. Two candidate genes located on chromosome 21, *HMGN1* and *DYRK1A*, have received the most attention. The protein product of *HMGN1* is a nucleosome-binding protein implicated in histone H3 modifications and it has been shown to alter B-cell development in Ts1Rhr mice [reviewed in 1]. Epigenetic changes related to *HMGN1* expression may lead to B cell proliferation

and leukemogenesis. *DYRK1A*, a member of the CMGC superfamily of protein kinases and located in the Down syndrome critical region (DSCR) of chromosome 21, is also one of the candidate genes implicated in DS-ML. The protein product of *DYRK1A* is essential for lymphoid, but not myeloid, development where it is involved in cell cycle regulation and appears to be important for shifting lymphocytes from a proliferative to a quiescent state [59].

iAMP21

Intrachromosomal amplification of chromosome 21 (iAMP21) occurs in 2% of non-DS pediatric ALL and has similarities to DS-ALL, further supporting the contribution of constitutional trisomy 21 to the pathogenesis of DS-ALL [reviewed in 1]. Many of the genes amplified in iAMP21 are the same genes implicated in constitutional trisomy 21, namely, *RUNX1* and miR-802, and possibly *DYRK1A* and *ETS2*. Other molecular abnormalities observed in the iAMP21 group include *IKZF* deletions (16%), *PAX5* deletions (8%), *CDKN2A* deletions (13%), *ETV6* deletions (15%), gain of X-chromosome (20%), and *P2RY8-CRLF2* fusions (17%). Among these, gain of X-chromosome and *P2RY8-CRLF2* fusions have been reported in DS-ALL in 24% and 22% of cases, respectively. A notable difference between the iAMP21 group and DS-ALL is the lack of *JAK2* mutations in the former.

Prognosis and Therapy

Higher relapse rates and overall inferior outcomes are observed in DS-ALL compared to the general population. While different biologic, cytogenetic, and molecular profiles may certainly contribute to this trend, individuals with Down syndrome appear to have a higher incidence of methotrexate toxicity, which has led to dose-adjusted protocols in this population [1, 60]. It has been suggested that the higher relapse rates are at least partially attributable to the difference in treatment protocols, which appear necessary due to the excessive morbidity and mortality observed with conventional protocols designed for the general pediatric population [1]. *JAK2* mutations are associated with high-risk features and relapsed disease in non-DS-ALL but apparently not in DS-ALL, while *KRAS* mutations were associated with poor outcome for DS-ALL in one study [57].

Hematopoietic stem cell transplantation is an option for high-risk or relapsed ALL in the general population, but data for DS-ALL is limited [1]. While targeted therapy does not yet play a major role, several new agents have been proposed as potential therapies in the DS and non-DS ALL populations, especially in the relapsed/refractory disease setting. Such agents include chimeric antigen receptor T cells, the CD19/CD3 bispecific antibody blinatumomab that redirects cytotoxic T cells to CD19-expressing leukemic cells, and agents targeting JAK2 (i.e., ruxolitinib, momelotinib) or mTOR (i.e., temsirolimus, everolimus) pathways [1].

Conclusions

There is an increased risk of developing ALL in individuals with Down syndrome. The molecular pathogenesis of DS-ALL involves three predominant molecular aberrations – *CRLF2* overexpression, *JAK2* alterations, and *RAS* mutations, with likely contributions from constitutional trisomy 21. Inferior outcomes are seen in DS-ALL when compared to the general population. While the WHO 2016 does not recognize DS-ALL as a distinct entity, the different clinical, cytogenetic, and molecular characteristics underscore the complexity and differences among DS-ALL and ALL in general pediatric population.

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