Down's syndrome as a model of a pre-leukemic condition

Shai Izraeli

Department of Pediatric Hemato-Oncology, Cancer Research Center, Safra's Children's Hospital, Sheba Medical Center, Sackler Faculty of Medicine, Tel-Aviv University, Israel. E-mail: sizraeli@sheba.health.gov.il

Up to 10% of children with Down's syndrome (DS) are born with an unusual clonal megakaryocytosis syndrome commonly called *transient myeloproliferative disorder* (TMD) or *transient abnormal myelopoiesis* (TAM) or *transient leukemia*. As suggested by the different names the disorder is usually transient and resolves spontaneously within up to several months. The biological mechanism of the spontaneous resolution is unclear. About 20% of these patients will, however, develop full blown malignant acute megakaryoblastic leukemia (AMKL) during their first 4 years of life.[1] In fact, the risk of AMKL is about 600 times higher in children with DS.[2] The factor(s) underlying the transformation from *benign* TMD into *malignant* AMKL are largely unknown. Acquired additional chromosomal abnormalities such as trisomy 8 and activating mutations in the JAK3 kinase may be associated with disease progression.[1,3]

GATA1 - a surprising participant

The peculiar association between DS and childhood megakaryoblastic disorders has led to an intensive search for a leukemogenic gene or genes on chromosome 21. A surprising twist in this story came with the discovery that a gene on chromosome X, *GATA1*, was mutated in the megakaryoblasts from all patients with DS and either TMD or AMKL.[4-6] The mutations occur before birth and they were also found in fetal liver of aborted DS fetuses.[7,8] The mutations are acquired as they are not found in remission samples, and are specific to the megakaryoblastic disorders associated with trisomy 21. *GATA1* encodes a zinc-finger transcription factor that regulates the normal development of the erythroid, megakaryocytic and basophilic/mast cell lineages.[9] Two isoforms of GATA1 are usually detected: a full length GATA1 translated from the first ATG on exon 2, and a shorter form (GATA1s) that is initiated from an ATG on exon 3. The normal function of GATA1s is unknown. Presumably the balance between these two products serves a regulatory function in normal megakaryocytic development. All the acquired mutations in the megakaryoblastic disorders of DS result in elimination of the full length GATA1 and the preservation of GATA1s.

Thus a clear model for multistep leukemogenesis in DS emerges (Figure 1A): in a relatively high proportion of DS patients, acquired mutations in *GATA1* are selected *in utero* and are probably responsible for the differentiation arrest and the initiation of clonal proliferation of immature megakaryoblasts. These mutations are necessary but insufficient for the development of the full blown AMKL that affects some of these patients during early childhood.

The collaboration between gene(s) on chromosome 21 and mutated GATA1 in megakaryocytic malignancies of DS is unique in its intrauterine occurrence and in its putative initiating role of a common and generally reversible clonal hematopoietic proliferation syndrome. At least three fascinating questions can be considered:

(i) Why do all the selected mutations result in the formation of the short isoform of GATA1? Does this isoform have a dominant pro-leukemogenic effect?

(ii) Why do GATA1 mutations and the megakaryoblastic proliferation occur only *in utero*? Germline trisomy 21 exists in all hematopoietic precursors throughout life in DS. *GATA1*-dependent megakaryopoiesis in the bone marrow also continues throughout post-natal life. So why does the selection of mutations in *GATA1* in DS patients occur only in the fetal liver?

(iii) What is the gene or genes on chromosome 21 that, when existing in one additional copy, select for the cells carrying the GATA1s mutation? What is the mechanism of this selection?

A clue to the answer to the first two questions comes from a recent study from the laboratory of Stuart Orkin.[10] Knock-in of the mutated *GATA1s* into the *GATA1* locus surprisingly resulted in normal adult megakaryopoieis. However, examination of the fetal liver revealed abundant proliferation of megakaryocytic progenitors. Orkin and his colleagues proposed the existence of a fetal hematopoietic progenitor that is sensitive to a dominant pro-proliferative effect of GATA1s. The presence of trisomy 21 enhances the survival and proliferation of these fetal cells resulting in a congenital pre-leukemia syndrome. Gene expression profiling of DS megakaryocytic leukemias[11] provides further support for a dominant pro-proliferative role for the GATA1s mutation. Recently a pedigree with a germ line GATA1s mutation has been reported.[12] These patients do not develop leukemia. Thus both trisomy 21 and GATA1s mutation are necessary for the congenital pre-leukemic condition.

The role of trisomy 21 – a developmental model

What is the gene (or genes) on chromosome 21 that promotes proliferation and provides a survival advantage to cells that acquire mutations in *GATA1*, a gene on chromosome X? The strongest candidate has been *RUNX1*[13,14] (also known as *AML1* or *CBFA2*). RUNX1 is a transcription factor that is required for normal

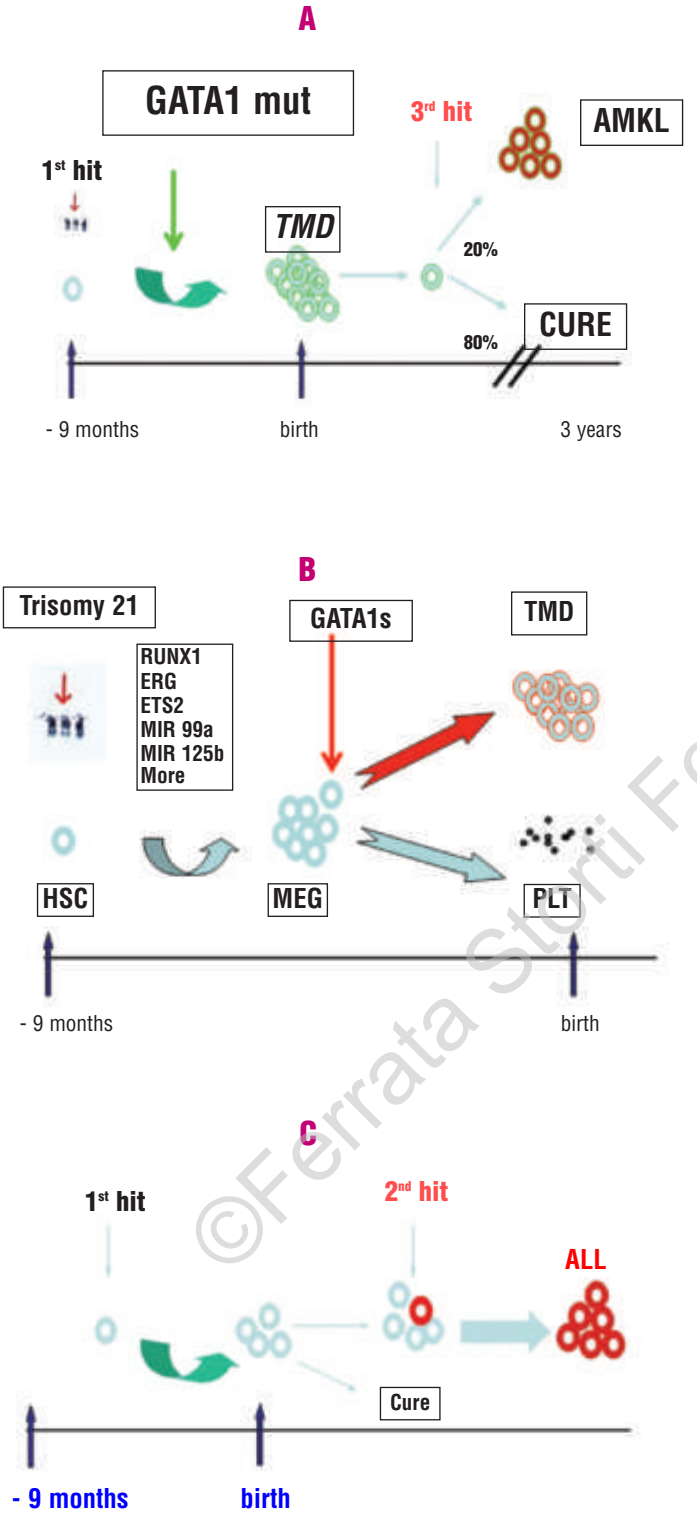

Figure 1. Models for leukemia development: 1A. The leukemia of DS – Acquired *GATA1* mutation during fetal liver hematopoiesis in cells carrying a germline trisomy 21 results in transient congenital leukemic proliferation TMD. An additional postnatal event is required for the development of full blown acute megakaryocytic leukemia (AMKL); 1B. *Rush-hour traffic jam* developmental model for the megakaryoblastic malignancies of children with Down's syndrome. Extra copies of several genes create a pro-megakaryopoiesis developmental pressure during fetal hematopoiesis. This, results in thrombocytosis in infants with Down's syndrome. Acquired mutation in *GATA1* (GATA1s) causes a *traffic jam* by blockade of platelet maturation coupled with enhanced proliferation of megakaryoblstic precursors leading to thrombocytopenia and congenital leukemia; 1C. Multistep pathogenesis of sporadic childhood leukemia (Greaves hypothesis) – a prenatal acquired genetic event (e.g. chromosomal translocation, hyperdiploidy) creates a large submicroscopic prenatal clone evident at birth. In most instances this clone will disappear, unless a postnatal genetic hit will cause its progression to full blown leukemia... HSC: hematopoietic stem cell; MEG: megakaryocytic progenitors; Plts: platelets.

hematopoiesis. It is commonly mutated and involved in various translocations in both myeloid and lymphoid leukemias. Most of these abnormalities cause *loss* of function. The surprising observation that the levels of full length RUNX1 are *lower* in DS AMKL despite the presence of trisomy 21, while the level of dominant negative runx1 isoform is unchanged[11,14] is consistent with a tumor suppressive role of RUNX1. Finally, RUNX1 abnormalities have generally not been detected in AMKL and, except for a single case, mutations in

RUNX1 have not been found in AMKL associated with DS. Therefore, at first sight, it seems that *RUNX1* is not the gene responsible for the leukemogenic activity of trisomy 21. Could an extra copy of *RUNX1* contribute to the evolution of pre-leukemia in DS, despite its general tumor suppressive properties?

Apparently, *RUNX1* plays a major role in megakaryocytic differentiation in a dose-dependent manner. Inherited mutations in *RUNX1* causing haplo-insufficiency with a low level of expression in hematopoietic stem cells lead to a syndrome of familial thrombocytopenia and increased susceptibility to leukemia. This rare human syndrome and other functional studies[15,16] suggest that *RUNX1* regulates megakaryopoiesis. It is therefore reasonable to hypothesize that an extra copy of *RUNX1* may enhance megakaryopoiesis.

Careful examination of the relatively small chromosome 21 reveals additional genes regulating megakaryopoiesis. We have recently demonstrated the potential involvement of *ERG*, an ets transcription factor on chromosome 21q, in megakaryopoiesis and megakaryocytic leukemias.[17] *ERG* is a proto-oncogene that is rarely involved in AMKL caused by the *ERG-TLS* fusion translocation. Increased expression of *ERG* was also identified as an independent bad prognostic factor in acute myeloid leukemia with normal karyotype.[18] Similar involvement in megakaryopoiesis has been suggested for the proto-oncogene *ETS2* as well as for at least one microRNA coding gene, miR 99a, and possibly also *MiR125b*.[19] Another chromosome 21 gene, *BACH1*, blocks terminal megakaryocytic differentiation.[20] Thus multiple genes on chromosome 21 are involved in megakaryopoiesis.

These observations led us to propose a developmental *rush hour–traffic jam* model to explain the role of trisomy 21 in the occurrence of megakaryocytic leukemias in DS[17] (Figure 1B). According to this model, trisomy 21 causes increased expression of these genes in fetal hematopoietic progenitor cells and creates a positive pressure towards megakaryopoiesis, in analogy to traffic towards downtown during rush hour. The GATA1s mutation is similar to a *traffic accident* – it prevents megakaryopoiesis from reaching the target (platelet formation) and further enhances the proliferation of a putative fetal megakaryocytic precursor, as shown by Orkin *et al.*[10] The mutation in *GATA1* downregulates the expression of *RUNX1* and increases the expression of *BACH1*[11] leading to a further block of megakaryocytic differentiation. The consequence is a pile-up of megakaryocytic precursors. In contrast to the car traffic-jam, only megakaryocytic progenitors with the GATA1s accumulate. This clonal accumulation results in the congenital pre-leukemic phenotype.

Two predictions may be made from this model. First that examination of fetal livers from DS embryos lacking GATA1s mutation will reveal enhanced megakaryopoiesis. Striking unpublished observations by Roberts *et al.* confirm this prediction (*I. Roberts, personal communication*). The second prediction is that the enhanced fetal megakaryopoiesis may lead to thrombocytosis at birth. Indeed normal DS infants have significantly higher platelets counts during the first 6 months of life.[21]

RUNX1, NCAM and non-cell autonomous defects?

In this issue Claudia Langebrake and colleagues report the interesting observation of a high percentage on NCAM (CD56)-positive myeloid cells in DS patients with AMKL during recovery from chemotherapy.[22] The same cells express high levels of RUNX1. As concomitant expression of RUNX1 and NCAM was also reported in ischemic hearts and both are expressed in NK cells, it was suggested that NCAM may be a target of RUNX1.[23] This hypothesis has yet to be verified experimentally. Significantly, the recovering hematopoietic cells after chemotherapy in DS patients do not harbor the GATA1s mutation.[5] Thus the important novelty of the report by Langebrake *et al.* is the demonstration of a significant increase in NCAM expression in non-leukemic cells of DS patients during stress hematopoiesis. Because the trisomy 21 in DS is constitutional, i.e. it resides in every cell, it may promote leukemia in a non-autonomous, indirect manner. Possibly, the presence of trisomy 21 in fetal *stromal* cells may change the micro-environment and support the proliferation of the special fetal hematopoietic progenitors that are sensitive to GATA1s. This hypothesis may also explain why the transient megakaryoblastic proliferation resolves after birth, as the special fetal microenvironment no longer exists. Interestingly, NCAM expression has been recently shown to contribute to the hematopoiesis-supporting capacity of a stromal cell line.[24] Thus the observation of Langebrake *et al.* raises a very interesting question: could the increased expression of RUNX1 in patients with DS enhance the expression of NCAM on fetal stromal cells thereby contributing to the tilt towards fetal megakaryopoiesis? As NCAM is also involved in immune regulation, could an abnormal regulation explain some of the immune deficiencies characteristic of DS? These fascinating questions should be addressed experimentally.

DS and acute lymphoblastic leukemia

The megakaryocytic leukemia of DS is a unique disease.[25] However patients with DS are also at a markedly increased risk of childhood acute lymphoblastic leukemias (ALL). Because tri- and tetrasomy of chromosome 21 are the most common acquired chromosomal abnormalities in ALL, the study of DS ALL may have direct implications for sporadic childhood ALL. In most published multi-institutional ALL protocols, patients with DS ALL account for about 1-3% of all cases.[26,27] The age distribution and the immunophenotype are

similar to those of *common ALL*. Common ALL is a B-cell precursor leukemia that occurs most frequently in young, pre-school children.

ALL may be caused by a direct oncogenic effect of trisomy 21, similarly to the role of additional chromosomes 21 in sporadic leukemias. Alternatively the effect of trisomy 21 may be developmental. As in the suggested model for the megakaryocytic leukemias of DS, constitutional trisomy 21 may enhance the proliferation of a normal fetal lymphoid progenitor. This excess proliferation could evolve into leukemia if additional genetic events occur. Viral infections and the immunological response have long been suggested to have a role in the pathogenesis of childhood common ALL[28,29] The markedly increased risk of ALL in DS could also be caused by the altered immunological environment and the increased infection rate that characterize DS. As suggested above, the observation of Langebrake *et al.*[22] on the expression of regulatory adhesion molecules such as NCAM may also be relevant for the evolution of ALL in DS patients.

Molecular epidemiology studies may clarify the leukemogenic role of constitutional trisomy 21. Common sporadic childhood ALL is usually associated with one of two genetic abnormalities: a structural chromosomal anomaly – fusing the *AML1* (*RUNX1*) gene on chromosome 21 with the *TEL* (*ETV6*) gene on chromosome 12, or a numerical abnormality – hyperdiploidy. These two genetic aberrations are mutually exclusive suggesting that each activates an oncogenic pathway leading to B-cell precursor leukemia. If trisomy 21 enhances the risk for childhood ALL indirectly we could expect a similar rate of secondary aberrations (hyperdiploidy or TEL/AML1 translocation) to that in sporadic common ALL. If, on the other hand, constitutional trisomy 21 has a direct leukemogenic effect similar to the role of the acquired extra copies of chromosome 21 in hyperdiploid ALL, then we would expect a *lower* prevalence of TEL/AML1 or hyperdiploid genotypes in the ALL of DS. Published studies are inconclusive and only a large international molecular epidemiological study of DS ALL could clarify this issue.

General implications

Recent studies have shown that most, if not all, childhood leukemias arise during fetal hematopoiesis.[30] Thus, similar to DS leukemias, sporadic childhood leukemias evolve in a multistep process. A primary genetic event (*first hit*) is acquired *in utero*. This event results in the formation of a preleukemic clone that can be detected at birth by molecular techniques. As in TMD, this clone regresses spontaneously in almost all children. Additional post-natal genetic events in the residual preleukemic cells are necessary for generation of acute leukemia, which occurs in a small fraction of these children (Figure 1C). Thus, studies on the mechanism of TMD regression and on the nature of events leading to full blown AMKL in DS are relevant for the general understanding of the process of leukemogenesis.

Trisomy or tetrasomy 21 is one of the most common abnormalities in sporadic leukemias. We have recently observed that chromosomal aneuploidy can be reliably identified from the gene expression signature because it results in mild overexpression of multiple genes from the extra chromosomes.[31] This finding is consistent with the developmental model for the evolution of leukemia in DS (Figure 1B). It assumes that trisomies cause the coordinated increased expression of several genes involved in leukemogenesis. DS leukemias are, therefore, a prime model for studying the role of aneuploidy – one of the fundamental questions in carcinogenesis research.

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