Cancer is a genetic disease of the somatic cell. Virtually all cancers, including leukemias, harbor multiple acquired genetic abnormalities. These abnormalities are commonly manifested by growth chromosomal abnormalities. These abnormalities can be either structural or numerical. Aneuploidy, change in the normal chromosomal copy number, is one of the frequent abnormalities in cancer. For example high hyperdiploid acute lymphoblastic leukemia (ALL) is the most common leukemia observed in young children. The leukemic cells contain extra copies of multiple chromosomes. The chromosomes involved are non-random. Chromosomes 21, X, and 6 are almost always involved, as are chromosomes 4, 10, 17 and occasionally 18. This specific leukemia syndrome suggest a causative role for the chromosomal numerical aberrations. Yet it the mechanisms by which acquired trisomies contribute to leukemia is unknown. Children with Down Syndrome (DS) are born with a 20-fold risk and about 600 risk for acute megakaryoblastic leukemia (AMKL) during their first four years of life that will not regress without chemotherapy. In fact, the risk of AMKL is about 600 times higher in children with DS. The factor(s) underlying the transformation from “benign” TMD into “malignant” AMKL are largely unknown.

Thus the megakaryocytic malignancies of DS provide a “natural genetic model” of multistep lineage specific leukemogenesis. Both the congenital disorder and the full blown AMKL are characterized by differentiation arrest of the megakaryocytic lineage. The marrow and the liver of infants with TMD contain a large number of dysplastic micromegakaryocytes. Identical cells are observed in the early stages of the AMKL of DS. Both disorders are also characterized by thrombocytopenia, indicative of poor platelets formation by the dysplastic megakaryocytes.

The peculiar association between DS and childhood megakaryoblastic disorders has led to intensive search for gene or genes on chromosome 21 than may cause the differentiation arrest and initiate the leukemia. A surprising twist in this story came with the discovery that a gene on chromosome X, GATA1, was mutated in the megakaryoblasts from all the patients with DS and either TMD or AMKL. The mutations were also found in fetal liver of aborted DS fetuses. The mutations are acquired as they are not found in remission samples, and are specific to the megakaryoblastic disorders associated with trisomy 21. No GATA1 mutations were found in other AMKLs, in sporadic acute myeloid leukemia (AML) or in the acute lymphoblastic leukemia (ALL) associated with DS. Thus a clear model for multistep...
leukemogenesis in DS emerges: 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 affect some of these patients during early childhood.

GATA1 encodes a zinc-finger transcription factor that regulates the normal development of the erythroid, megakaryocytic and basophilic/mast cell lineages. Mice lacking GATA1 expression in the megakaryocytic lineage have thrombocytopenia and extensive proliferation of immature megakaryoblasts. Inherited inactivating mutations in GATA1 in humans cause a familial dyserythropoietic anemia and thrombocytopenia. Thus GATA1 normally suppress the proliferation of megakaryocytic and erythroid precursors while promoting their differentiation.

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. GATA1s is lacking the amino-terminal transactivation domain and is therefore less active than full length GATA1. 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. GATA1s is less active in promoting megakaryocytic differentiation and, therefore, less mature, abnormal, megakaryoblastic precursors accumulate.

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. What is/are the gene/genes on chromosome 21 that promote(s) proliferation and provide(s) survival advantage to cells that acquired mutations in GATA1, a gene on chromosome X? The strongest candidate has been RUNX1 (also known as AML1 or CBFA2). RUNX1 is a transcription factor that is required for normal hematopoiesis. It is commonly mutated and involved in various translocations in both myeloid and lymphoid leukemias. Inherited mutations in RUNX1 causing haploinsufficiency with low level of expression in hematopoietic stem cells cause a syndrome of familial thrombocytopenia and increased susceptibility to leukemia. However, RUNX1 abnormalities have generally not been detected in AMKL and, except for a single case report, mutations in RUNX1 have not been found in AMKL associated with DS. A recent study from the Goldfarb’s laboratory has demonstrated that RUNX1 directly interacts with GATA1 and that this interaction is important for megakaryopoiesis (Elagib et al. Blood 2003;101: 4333–41). Surprisingly, GATA1 transactivation domain was shown to be critical for this interaction. Thus one possibility is that the elimination of that domain in GATA1s provides “two hits in one event” — the preservation of a hypoactive isoform of GATA1 that block differentiation and the formation of abnormal complexes of RUNX1, lacking GATA1, that, plausibly, promote survival and proliferation of these cells. This speculation has to be experimentally tested, as is the question of how an excess of one allele of RUNX1 in trisomy 21 or a potential dysregulation of RUNX1 expression, predispose for this unique collaboration with mutated GATA1.

We have recently demonstrated the potential involvement of ERG, an ets transcription factor on...
chromosome 21q (Rainis et al., Cancer Research 2005, in press). ERG is a protooncogene that is rarely involved in AMKL caused by the ERG-TLS translocations. We have shown that it is expressed in CD34 cells, in normal megakaryocytes and platelets, in megakaryocytic leukemias (whether or not associated with DS) but not in normal or malignant erythroblasts. ERG was induced upon megakaryocytic differentiation of erythroleukemia cells. Forced expression of ERG in the erythroleukemia cell line caused a phenotypic shift from the erythroid into the megakaryocytic lineage. Thus ERG seems to be a positive regulator of normal and malignant megakaryopoiesis.

I propose the “rush hour traffic jam model” for the occurrence of megakaryocytic leukemias in DS (see Figure). Extra copies of several genes on chromosome 21 (including ERG, RUNX1, probably ETS2 and possibly some others) create a positive pressure towards megakaryopoiesis, similarly to the traffic pressure towards downtown during rush hour. Supporting this suggested enhanced megakaryopoiesis is the observation that normal DS infants have significantly higher platelets counts during the first six months of life (Kivivuori Clin Genet 1996;46:15–19).

The GATA1s mutation is similar to a “traffic accident” – it prevents megakaryopoiesis from reaching the target – platelet formation. 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 leukemic phenotype.

What are the general implications of DS megakaryocytic leukemias? First, it is a prime example of collaborating pro-proliferation and differentiation arresting mutations in leukemia. Second, it may provide a general model for the role of aneuploidy in leukemia. Unique to whole chromosome aneuploidy is the parallel amplification of multiple genes which may act cooperatively in the same leukemogenic pathway. Finally, deciphering the regulatory factors working towards the selection of GATA1s progenitors during fetal liver hematopoiesis in DS may be relevant to sporadic childhood leukemia since recent studies have clarified that most, if not all, childhood leukemia arise during fetal hematopoiesis.

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References