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The impact of trisomy 21 on early human hematopoiesis

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Although children with Down syndrome (DS) are not cancer-prone in general, they have a 150-fold increased risk of acute myeloid leukemia of DS (ML-DS) and a 33-fold increased risk of B-cell acute lymphoblastic leukemia (B-ALL).[1] In virtually all cases of ML-DS, but not in other leukemias, the leukemic cells acquire N-terminal mutations in the *GATA1* gene, a key hematopoietic transcription factor and have an erythro-megakaryoblastic phenotype.[2,3] The same N-terminal *GATA1* mutations are present in the clonally related, neonatal preleukemic disorder transient abnormal myelopoiesis (TAM), which is unique to DS and often precedes ML-DS.[2] The identification of *GATA1* mutations in ML-DS and TAM, and their unique association with DS and trisomy 21 (T21) (*GATA1* mutations are not leukemogenic in the absence of T21), has provided an exciting and potentially tractable model of human myeloid leukemogenesis and, more generally, the impact of aneuploidy on human hematopoiesis.

T21 is likely to impact on hematopoietic cell biology in multiple complex ways. Several genes on chromosome 21 (Hsa21), such as *RUNX1*, *ERG* and *DYRK1A,* encode proteins or microRNAs, such as miR-125b, with relevant functions in hematopoietic cells. However, while trisomic genes, individually or collectively, may be directly involved through gene dosage either in a hematopoietic cell-autonomous fashion or via other cell types, the effects may also be exerted indirectly via disomic genes. To address this, several investigators have studied mouse models of DS.[4] Although these models implicate deregulated expression of Hsa21-encoded genes as tumor-promoting, most evidence suggests that the mouse may not be a suitable model.[4] Critically, none of the models spontaneously develop TAM and/or ML-DS. Furthermore, the hematopoietic phenotype of germline N-terminal *GATA1* mutations in disomic humans[5] is markedly different to mouse.

Adopting an alternative approach to investigating the role of T 21 gene dosage, we set out to determine the cellular consequences of T21 in primary human fetal and neonatal hematopoietic cells, prior to acquisition of *GATA1* mutations. We,[6] and others,[7] found specific and marked expansion of megakaryocyte-erythroid progenitors (MEP) and proliferative abnormalities of common myeloid progenitors (CMP) in DS fetal liver (FL) in the absence of detectable *GATA1* mutations. These observations have now been supported by work in human T21 embryonic stem (ES) and induced pluripotent stem (iPS) cells that illustrate arrested erythroid-megakaryocyte progenitor/precursor differentiation both of embryonic[8] and fetal stages of hematopoiesis.[9]

To investigate whether the abnormalities in T21 FL were confined to MEP/CMP or extended to the hematopoietic stem cell (HSC) or multipotential progenitor (MPP) level, we recently performed detailed immunophenotypic and functional analysis of the HSC/MPP, committed myeloid and B-lymphoid compartments of human T21 FL without *GATA1* mutations and compared these with normal human FL.[10] We demonstrated for the first time that in human FL, T21 itself increases immunophenotypic HSC, clonogenicity and MK-erythroid output and biases erythroid-megakaryocyte primed gene expression with associated MEP expansion. In addition, immunohistochemical studies of T21 FL sections showed that megakaryocytes were both increased[10] and abnormal (G. Cowan, unpublished data). Furthermore, we found severe impairment of B-lymphoid development, with ~10-fold reduction in pre-pro B-cells and B-cell potential of HSC, in tandem with reduced HSC lymphoid gene expression priming.[10]

These data support the notion that an extra copy of Hsa21 in FL HSC is sufficient to perturb their growth and differentiation. This in turn would lead to an increased FL MEP compartment and, following acquisition of *GATA1* mutation(s), to a selective expansion of a mutant erythro-megakaryocytic leukemic blast cell population manifesting as the clinical condition TAM in late fetal, or early neonatal life (**Fig. 1**).

What our studies did not explain was whether the perturbation of hematopoiesis in T21 FL was dependent on specific supportive interactions with the FL microenvironment or, alternatively, was entirely hematopoietic cell-autonomous. Preliminary data show that while normal FL HSC reliably sustain multilineage bone marrow (BM) engraftment in adult immunodeficient (*NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}*/SzJ; NSG) mice, T21 FL HSC engraft adult murine BM very poorly (G. Cowan, unpublished data), implicating a crucial role for the FL microenvironment. On the other hand, where T21 FL cells did engraft, the HSC/MEP expansion and B-lymphoid deficiency of primary FL cells was maintained. Together these data support a model in which both cell-autonomous effects of T21 and the specialized

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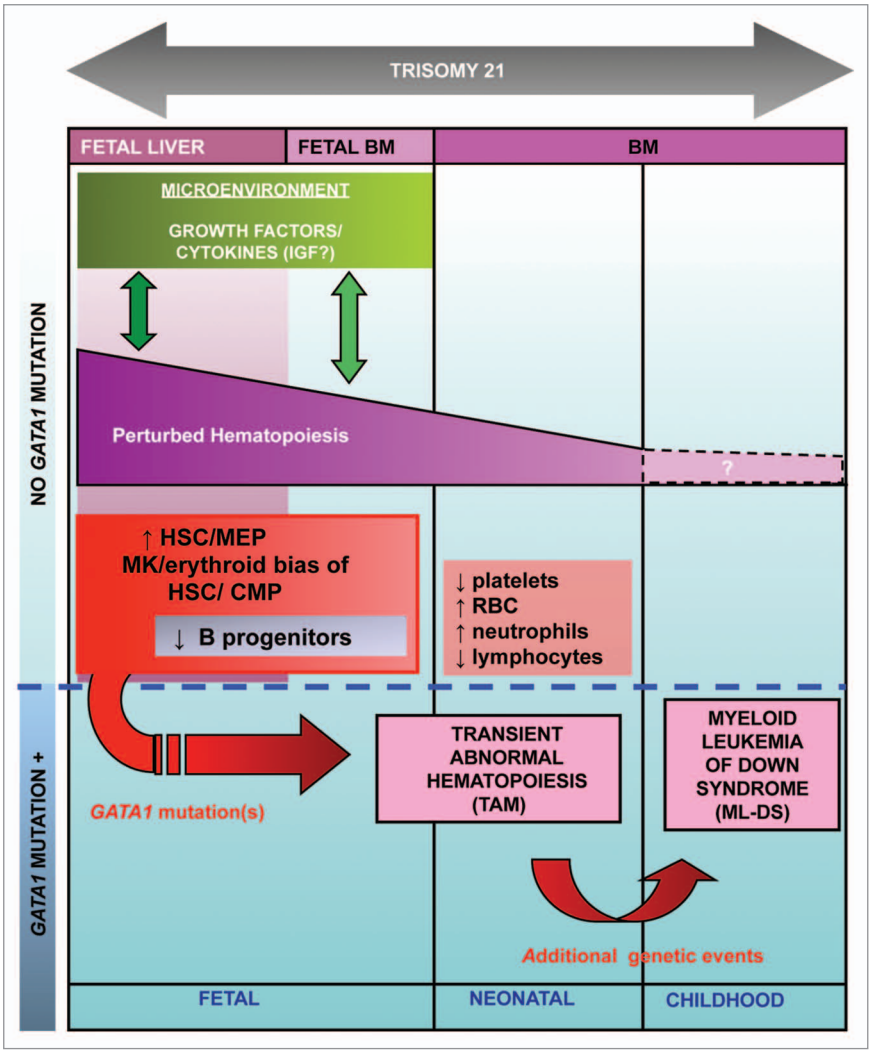

Figure 1. Impact of trisomy 21 on fetal and post-natal hematopoiesis. Schematic representation of molecular, biologic and clinical data, summarizing the effect of trisomy 21 (T21) on fetal, neonatal and childhood hematopoiesis. Fetal liver and, to a lesser extent, fetal bone marrow (BM) trisomic for chromosome 21 demonstrate perturbed hematopoiesis with an expansion of the hematopoietic stem cell compartment (HSC) and megakaryocyte-erythroid progenitors (MEP) and reduced B lymphopoiesis, even in the absence of *GATA1* mutations. Interaction of hematopoietic cells with the T21 fetal liver and/or BM microenvironment may play a crucial role in initiating abnormal fetal hematopoiesis. Subsequent acquisition of *GATA1* mutations in the abnormal/expanded T21 fetal liver HSC and progenitors results in transient abnormal myelopoiesis (TAM) in late fetal/neonatal life. Although most cases of TAM resolve spontaneously; in 15–30% of cases, additional genetic/epigenetic events lead to Down syndrome-associated acute myeloid leukemia (ML-DS) before the age of 5 y. Abnormalities in hematopoiesis are likely to persist in childhood, but detailed systematic studies are necessary to establish this.

fetal hematopoietic microenvironment are necessary to drive abnormal hematopoiesis in DS. Consistent with this, we have now found an increase in MEP and clonogenic megakaryocyte progenitors in T21 human fetal BM, although to a lesser extent than in FL, and there is trilineage perturbation of neonatal hematopoiesis. Importantly, B-lymphoid progenitors were also reduced in T21 fetal BM compared with normal gestation-matched controls (A. Roy, unpublished data) suggesting that molecular resetting of the fetal B-lymphoid program may contribute to B-cell immune deficiency and B-ALL in children with DS.

In conclusion, recent data from primary human FL,[10] as well as fetal BM, ES cells and iPS,[8,9] indicate that T21 itself alters human fetal HSC and progenitor biology, causing multiple defects in lympho-myelopoiesis. These data provide clues to possible mechanisms through which T21, or aneuploidy in general, may perturb hematopoietic cell growth and differentiation and a model with which to investigate these. However, the molecular basis through which T21 exerts these effects is likely to be extremely complex, to be both tissue- and lineage-specific and to be dependent on the FL, and possibly fetal BM, microenvironment, analogous to the role of the specialized tumor microenvironment in enabling and sustaining neoplastic cancer cells.

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