host was actually in a long-term cold environment may well have favorable bioenergetic effects in overweight individuals.

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buds and is indistinguishable from acute leukemia. The majority of infants survive, and the condition resolves spontaneously by 3 months of age. However, about 20% of newborns diagnosed with TMD go on to develop acute megakaryoblastic leukemia (AMKL) before 4 years of age. Fortunately, the majority of these patients can be cured with chemotherapy.

Virtually all the TMD and AMKL cells isolated from individuals with DS have been found to carry an acquired mutation in the gene on chromosome X that encodes the transcription factor GATA1. While mutations differ among patients, and occasionally more than one mutation is identified in the same patient, in all instances they result in the generation of a short GATA1 protein lacking the N-terminal domain (GATA1s). GATA1 is a critical regulator of the megakaryocytic and erythroid lineage, and, in the presence of GATA1s, megakaryocytic differentiation is impaired and the proliferation of fetal megakaryocytic precursors is enhanced, possibly via activation of the transcription factor E2F. Progression of TMD to AMKL is associated with the acquisition of heterogeneous additional mutations in genes such as JAK2, JAK3, MPL, and PS3, and additional chromosomal aberrations, resulting in aneuploidy.

The role of cT21 in promoting TMD and AMKL was a mystery until the publication of two independent breakthrough studies in 2008 (4, 5). Both teams of researchers analyzed fetal livers from human fetuses and discovered a dramatic cell-autonomous increase in the frequency of megakaryocytic-erythroid progenitors and the condition resolves spontaneously in the generation of a short GATA1 protein lacking the N-terminal domain (GATA1s). GATA1 is a critical regulator of the megakaryocytic-erythroid lineage and, in the presence of GATA1s, megakaryocytic differentiation is impaired and the proliferation of fetal megakaryocytic precursors is enhanced, possibly via activation of the transcription factor E2F. Progression of TMD to AMKL is associated with the acquisition of heterogeneous additional mutations in genes such as JAK2, JAK3, MPL, and PS3, and additional chromosomal aberrations, resulting in aneuploidy.

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Figure 1

Multistep pathogenesis of the megakaryocytic malignancies of DS. cT21 promotes cell-autonomous fetal megakaryo-erythropoiesis. Acquired mutation of GATA1, leading to expression of GATA1s, further enhances the proliferation of these precursors and blocks terminal megakaryocytic proliferation, manifesting at birth as TMD. TMD is transient; however, in 20% of the patients, a residual submicroscopic clone acquires additional genetic abnormalities, examples of which are discussed in the main text, that lead to AMKL, which is always diagnosed before 4 years of age.

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The subcellular localization of NFAT proteins depends on their phosphorylation status. Phosphorylated NFAT proteins are inactivated by translocation to the cytosol. Cyclosporine A (CsA) and FK506 are pharmacological inhibitors of the pathway, acting by blocking calcineurin. Pharmacological inhibitors of DYRK1A, such as harmine, activate the NFAT pathway.

specification during development and metastatic spread of epithelial cancers (16).

The NFAT proteins are the final messengers in a vertebrate-specific signaling cascade initiated by an increase in the concentration of intracellular Ca\(^{2+}\) in response to various external stimuli (e.g., T cell activation) (Figure 2 and ref. 15). The subcellular localization of NFAT proteins depends on their phosphorylation status. Phosphorylated NFAT proteins are cytosolic and hence transcriptionally inactive. Ca\(^{2+}\) binds to the calcium sensor calmodulin, which in turn binds and activates the protein phosphatase calcineurin. After dephosphorylation by calcineurin, NFAT proteins translocate to the nucleus, in which they cooperate with multiple transcription factors to regulate gene expression. Nuclear NFAT proteins are phosphorylated by DYRK1A, glycogen synthase kinase-3 (GSK3), and casein kinase 1 (CKI) and translocate back to the cytosol. DYRK1A is a “priming” kinase, as phosphorylation mediated by DYRK1A is required for subsequent NFAT phosphorylation by GSK3.

DYRK1A is one of two inhibitors of the NFAT pathway encoded by genes in the “DS-critical region” of the long arm of chromosome 21. The other is calcipressin 1, which binds and inhibits calcineurin and is encoded by RCAN1 (also known as DSCR1), itself a target of NFAT. Consistent with this, experimental and mathematical evidence suggest that the NFAT pathway is inhibited in cells with DS (14). Moreover, a pathologic effect of this inhibition is suggested by the fact that mice lacking NFAT factors or overexpressing DSCR1 have skeletal, neuronal, behavioral, and cardiac defects reminiscent of those observed in patients with DS. Individuals with DS also have impaired thymic function associated with immunodeficiency (17, 18). Interestingly, although children with DS have a markedly increased risk of B cell precursor ALL, T-ALL is extremely rare in individuals with DS (3). One may speculate that both the immunodeficiency and the rarity of T-ALL may be also related to inhibition of the NFAT pathway in DS.

Suppression of the NFAT pathway by the chromosome 21-encoded proteins DYRK1A and RCAN1 has also been proposed to explain the lower rate of invasive epithelial cancers in adults with DS (19). Paradoxically, the data generated by Malinge et al. (2) suggest that inhibition of NFAT proteins by DYRK1A promotes the megakaryocytic malignancies associated with DS. It is worthwhile to note that these investigators mainly showed that inhibition of the NFAT pathway correlated with overexpression of DYRK1A and therefore did not rule out the possibility that other substrates may mediate the leukemogenic activity of DYRK1A. Interestingly, another inhibitor of calcineurin, FKBP51, has been shown to be induced during megakaryocytic differentiation and to accumulate in idiopathic myelofibrosis, a megakaryocytic neoplasm (20). These independent observations provide strong support for the hypothesis that NFAT inhibition and megakaryocytic malignancies are linked.

The data generated by Malinge et al. (2) support the hypothesis that, while DYRK1A suppresses the growth of epithelial and lymphoid tumors, it is a megakaryocytic oncogene. A similar dual role has been proposed for another chromosome 21-encoded protein, RUNX1. RUNX1 is a tumor suppressor in both lymphoid and myeloid leukemias but has been suggested to be involved, as a result of its overexpression, in DS-AMKL (8).

Future perspective and challenges

Despite the progress made by Malinge et al. (2) and other researchers (8–10, 11, 12, 21) in deciphering single candidate genes that mediate the leukemogenic effect of cT21, it is still unclear how cT21 functions during the transition from preleukemia to TMD. How many genes on chromosome 21 participate in the leukemogenesis process, and what is the quantitative contribution of each gene? At what stage of development are they involved? What is the basis for the unique and specific cooperation between cT21 and the GATA1 mutation?

Chromosomal aneuploidies are commonly detected in cancer (22). Thus, the answers to these questions as well as additional studies of the role of cT21 in the leukemias associated with DS may have general implications for understanding the oncogenic mechanisms of acquired chromosomal aneuploidies.

A major experimental hurdle has been the lack of appropriate experimental models. Although Malinge et al. have nicely shown that DS-AMKL can be modeled in the mouse by introducing three hits, namely cT21, Gata1 mutation, and a mutation in Mpl (2), mouse models of DS are limited. For example, they do not have the fetal phenotype observed in human DS fetuses. DS-AMKL cell lines might not be appropriate for study, as they represent a stage occurring after either the preleukemic or TMD phase. Substantial progress has been made recently by the expansion of trisomic hematopoietic cells from DS fetuses in immunodeficient mice (4) and by the generation of induced pluripotent stem cells from DS.
fetal hematopoietic and TMD cells (23). Such experimental tools would allow systematic evaluation of human cT21 in the correct developmental context.

Whether DYRK1A is functioning through the NFAT pathway or another pathway in DS-AMKL, its inhibition may have therapeutic benefit. Malinge et al. provide a proof of concept that DYRK1A inhibitors may be clinically useful in the context of DS-AMKL by demonstrating that harmine, a small-molecule inhibitor of DYRK1A kinase activity, can inhibit the growth of megakaryoblastic leukemic cell lines with trisomy 21 (2). Thus, DYRK1A inhibitors may be a specific targeted therapy for DS-AMKL.

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