

Stem and progenitor cell dysfunction in human trisomies

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Abstract

Trisomy 21, the commonest constitutional aneuploidy in humans, causes profound perturbation of stem and progenitor cell growth, which is both cell context dependent and developmental stage specific and mediated by complex genetic mechanisms beyond increased Hsa21 gene dosage. While proliferation of fetal hematopoietic and testicular stem/progenitors is increased and may underlie increased susceptibility to childhood leukemia and testicular cancer, fetal stem/progenitor proliferation in other tissues is markedly impaired leading to the characteristic craniofacial, neurocognitive and cardiac features in individuals with Down syndrome. After birth, trisomy 21-mediated premature aging of stem/progenitor cells may contribute to the progressive multi-system deterioration, including development of Alzheimer's disease.

Keywords Down syndrome; hematopoietic stem cells; leukemia; neural progenitors; trisomy 21

DOI 10.15252/embr.201439583 | Received 14 September 2014 | Revised 16 November 2014 | Accepted 20 November 2014

EMBO Reports (2015) 16: 44–62

See the Glossary for abbreviations used in this article.

Introduction

Trisomy 21, trisomy 18 and trisomy 13 are the commonest constitutional trisomies in humans [1]. In contrast to trisomy 18 and 13, where fewer than 10% of affected children survive beyond the first year of life [2–4], median life expectancy for individuals with trisomy 21 (Down syndrome; DS) is around 60 years [5]. Most attention has focused on trisomy 21, not only because it is 20 times and 40 times more frequent than trisomy 18 and 13, respectively, but also because prolonged survival in DS suggests that most cells evolve epigenetic, transcriptional and/or translational regulatory mechanisms which allow them to adapt to the additional copy of chromosome 21 (Hsa21). To some extent, this may reflect the relatively low number of protein-encoding genes on Hsa21 (~240) (<http://www.ensembl.org/biomart/martview/>). However,

the characteristic phenotypic variability between different individuals with DS points to considerable complexity. Understanding the genomic determinants of this complexity continues to reveal fascinating insights relevant not only to DS, but also to aneuploidy in general.

Here, we review the impact of human trisomies on stem and progenitor cells. We will focus on trisomy 21, and particularly on hematopoiesis, where advances in techniques for characterization of highly purified primary cells and the development of induced pluripotent stem cell (iPSC) and animal models are beginning to answer some of the questions about the mechanisms by which trisomies cause human disease.

Phenotypic variability in constitutional trisomy 21 (DS)

DS is a multisystem disorder caused, in most cases, by meiotic non-disjunction of the maternal Hsa21, resulting in a third copy of the entire Hsa21 in all cells [6,7]. The clinical and biological impact of trisomy 21 nevertheless varies widely, not only between individuals with DS, but also in different tissues, the cell types within these tissues and at different ages [reviewed in 8–10]. Within this phenotypic variability, certain characteristics, such as the craniofacial abnormalities, hypotonia and cognitive impairment, are common to all individuals with DS (Table 1) and may therefore share temporal, biological or genetic mechanisms. Other features, such as cardiac defects or gastrointestinal anomalies, affect only a subset of patients and so may be more strongly influenced by inter-individual differences which interact with trisomy 21-driven changes, in heart and gut development, respectively, during embryogenesis. Many of these phenotypic abnormalities can be modeled using mouse segmental trisomies allowing the consequences of trisomy 21 to be investigated in an appropriate cellular context (Table 2).

The impact of age on the phenotypic expression of DS is increasingly recognized and, for many cells and tissues, is essential to consider in selecting the best experimental model to investigate the role of trisomy 21. Abnormalities of hematopoiesis begin in fetal life and have their maximal expression in the neonatal period when nearly all DS neonates have multiple hematologic defects, including 30% who develop a unique preleukemic syndrome confined to the first few months of life [11]. By contrast, the effects of trisomy 21 on

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Glossary

AICD	APP intracellular domain
ALL	acute lymphoblastic leukemia
AVSD	atrioventricular septal defect
BCP	B-cell progenitor
DS	Down syndrome
FDR	false discovery rate
GEDD	gene expression dysregulation domain
GEP	gene expression profile
GMP	granulocyte–monocyte progenitor
GSK3b	glycogen synthase kinase 3b
hESC	human embryonic stem cell
Hsa21	<i>Homo sapiens</i> chromosome 21
HSC	hematopoietic stem cell
HSPC	hematopoietic stem and progenitor cell
IGF	insulin-like growth factor
iPSC	induced pluripotent stem cell
MEP	megakaryocyte–erythroid progenitor
miR	microRNA
ML-DS	myeloid leukemia of Down syndrome
Mmu	<i>Mus musculus</i>
NFAT	nuclear factor of activated T cells
qPCR	quantitative polymerase chain reaction
Shh	sonic hedgehog
T21	trisomy 21
TAM	transient abnormal myelopoiesis
VEGF	vascular endothelial growth factor

visual and hearing impairment, thyroid function and cognitive function increase with age with progressive pathological changes in the brain in almost all DS individuals and clinical evidence of dementia in ~50% [12–19]. These age-related differences in phenotypic expression in DS suggest that trisomy 21, through patterns of gene expression which may be established early in development, causes premature, or accelerated, aging of a range of cell types. Evidence in support of this is now emerging [20], as discussed below.

Although several Hsa21 genes have been linked to the phenotypic expression of specific aspects of DS, such as leukemia and dementia, the mechanism(s) by which trisomy of individual genes or groups of genes contributes to the disorder remains unclear [reviewed in 8,9,21–24]. Investigators have used three broad approaches to investigate this question: mouse models trisomic for one or more of the genes on Hsa21, genomic association studies and comparative studies between human cells trisomic or disomic for Hsa21.

Mouse models of DS

The phenotypic characteristics of the most well-established mouse models of DS, and the extent to which they recapitulate the human phenotype, are summarized in Table 2. These include the only transchromosomal mouse model (Tc1) in which most of Hsa21 is present [25] albeit with several deleted or rearranged genes [26]. A number of more recent mouse mutants carrying genomic rearrangements of Hsa21 syntenic regions (on Mmu10, Mmu16 and Mmu17) that are trisomic for some, or most, of the ~250 mouse genes orthologous to Hsa21 genes have been described [27–31]. These interesting models, which may better mimic some aspects of human DS, have so far been used mainly to model the neurocognitive and

cardiac defects in DS [27–31]. Details of these, and of elegant refinements to narrow down the Hsa21 regions linked to defined phenotypes, are described in several reviews [32–42] and are only briefly discussed here in relation to their insight into the effects of trisomy 21 on stem/progenitor cells.

The impact of trisomy 21 on stem cell function

There is increasing recognition that trisomy 21 impacts on stem cell function in a number of ways (Fig 1). In hematopoiesis, for example, trisomy 21 affects the self-renewal, proliferation and differentiation of hematopoietic stem and progenitor cells (HSPC) either directly or via the hematopoietic microenvironment [43–52]. Studies in other tissue types, where stem and progenitor cells are often more difficult to identify and isolate, suggest that trisomy 21 also causes many of the defects in craniofacial, brain and cardiac development through perturbations of stem/progenitor cell growth and differentiation and altered interactions with microenvironmental and temporal cues. These alterations in stem/progenitor proliferation may underlie the increased susceptibility of some cell types, such as HSPC and primordial germ cells to malignant transformation [53–60] and of HSPC to premature aging in DS [20], as discussed in detail below.

Hematopoiesis and leukemia

The link between childhood leukemia and DS provides strong evidence for a particular susceptibility of hematopoietic cells early in life to perturbation of the normal mechanisms which control their growth and differentiation. Leukemias in DS have several unique features which hint at the ways in which trisomy 21 alters the behavior of HSPC [reviewed in 21,61,62]. First, the frequency of both myeloid leukemias and lymphoid leukemias is increased, by 150-fold and ~30-fold, respectively [53,59], indicating that trisomy 21 affects both myeloid and lymphoid progenitors. Second, these leukemias have a distinct temporal pattern of onset. Myeloid leukemia of DS (ML-DS) originates in fetal liver HSPC and presents either as a neonatal preleukemic syndrome known as transient abnormal myelopoiesis (TAM) or as full-blown ML-DS in children under the age of 5 years [21,61,62]. The peak age at presentation for acute lymphoblastic leukemia in DS (DS-ALL) is 1–4 years and, in contrast to ALL in individuals without DS, never presents in neonates or infants [53]. Third, leukemias in DS have distinct biologic and molecular features. Leukemic cells in ML-DS and TAM harbor N-terminal truncating mutations in the key hematopoietic transcription factor *GATA1*, which result in exclusive production of a short *GATA1* protein (Gata1s) with altered functional properties together with loss of expression of full-length *Gata1* since the *GATA1* gene is on the X chromosome [63–67]. Such mutations are not leukemogenic in the absence of trisomy 21 [68]. In DS-ALL, which in contrast to ALL in children without DS is always a B-precursor disease [69], ~60% of cases have aberrant expression of the CRLF2 receptor and around half of these have *RAS* mutations or mutations activating JAK-STAT growth-promoting signaling pathways [70–76].

Impact of trisomy 21 on fetal, neonatal and adult human hematopoiesis In contrast to most other tissues, hematopoietic tissues

Table 1. Phenotypic characteristics of Down syndrome.

Characteristic	Frequency (%)	Reference
Craniofacial Epicanthal folds Upward slanting palpebral fissures Flat nasal bridge Small brachycephalic head Small ears Small mouth	~100	[10, 114, 194, 195]
Other musculoskeletal abnormalities Hypotonia Single transverse palmar crease Clinodactyly with wide spacing	~100	[10, 196]
Cognitive impairment Reduced brain volume Learning and memory defects	~100	[10, 17, 197]
Dementia	40–50, increases with age	[12, 13, 14, 15, 16, 17, 18, 198, 199]
Visual	18–60, increase with age	[10, 18]
Hearing	18–80, increase with age	[10, 18]
Thyroid disease	1–54, increase with age	[10, 18, 196, 200, 201]
Cardiac defects ASD (45%) VSD (35%) Isolated secundum (8%) Isolated PDA (7%) Isolated Fallot's (4%) Other	40–50	[202, 203, 204]
Gastrointestinal defects	12	[10]
Benign hematological abnormalities Neonatal thrombocytopenia Neonatal polycythemia Neonatal neutrophilia, blast cells Macrocytosis	~100	[11]
Preleukemia and leukemia TAM and silent TAM Acute myeloid leukemia Acute lymphoblastic leukemia	30 1 1	[11, 53] [53] [53]
Non-hematologic cancers	50% of risk of individuals without DS	[18, 53, 56, 58, 59]

contain a well-characterized hierarchy of stem and progenitor cells, which can be readily isolated for molecular and functional studies. Characterization of the hematologic abnormalities in human DS therefore offers one of the best ways to understand how trisomy 21 perturbs cell biology and how cells adapt to aneuploidy. Recent studies in primary human fetal liver and neonatal cells [11,45], supported by data from human iPSC and hESC [46,47], demonstrate that trisomy 21 causes major disturbance throughout the entire hematopoietic hierarchy from HSC through to progenitors and mature cells (Fig 2). In particular, in fetal liver, trisomy 21 alters the balance of HSPC differentiation, promoting expansion and proliferation of megakaryocyte–erythroid progenitors (MEP) and megakaryocytes during the second trimester at the expense of both granulocyte–monocyte progenitors (GMP) and B-cell progenitors (BCP) [45]. There is also a 3.5-fold expansion in HSC numbers, and *in vitro* purified trisomy 21 fetal liver HSCs have erythroid–megakaryocyte-biased gene expression together with reduced

expression of lymphoid genes. Consistent with this, fetal liver HSC function is also markedly abnormal in DS. In particular, fetal liver HSCs generate more megakaryocyte and erythroid cells while their B-cell potential is severely impaired [45]. Since *GATA1* mutations were not detectable in these cells, these data indicate that trisomy 21 itself perturbs fetal liver hematopoiesis.

The effects of trisomy 21 on primary human fetal liver HSPC raise many questions. First, since these studies were confined to second-trimester fetal liver, it is not clear whether the effects are confined to this gestation. Interestingly, Chou *et al* [47] found that trisomy 21 iPSC differentiated under conditions designed to model yolk sac hematopoiesis showed enhanced erythroid, but not megakaryocyte, differentiation *in vitro*, suggesting the effects of trisomy 21 may be developmental stage specific. More recently, our group studied hematopoiesis in neonates with DS. In the presence of *GATA1* mutations, DS neonates developed the preleukemic condition, TAM. However, even in the absence of *GATA1* mutations, DS

Table 2. Mouse models of Down syndrome.

	Ts65Dn	Ts1Cje	Ts1Rhr	Tc1	Ts1Yeh;Ts2Yeh;Ts3Yeh Dp(16)1Yeh/+; Dp(10)1Yeh/+; Dp(17)1Yeh/+	Ts1Yah
Number of trisomic Hsa21 orthologs	~100 (also trisomic for ~60 genes on Mimu17 not syntenic for Hsa21)	~80	33	Transchromosomic (trisomic for 200 RefSeq Hsa21 genes*)	~175	~12
Craniofacial	Small mandible Brachycephaly	Small mandible	Large mandible	Small mandible	Normal appearance	Not reported
Learning and memory	Differences in face, palate-recapitulate human DS Impaired spatial learning and memory Impaired motor coordination	Differences in face, palate-recapitulate human DS Altered hippocampal dependent learning	Abnormalities do not recapitulate human DS Impaired novel object recognition	Defect in short-term memory and motor co-ordination	Recapitulates most of the behavioral features of Ts65Dn	Impaired novel object recognition but improved hippocampal-dependent spatial learning
Brain structure	Reduced brain volume Reduced cerebellar volume	Reduced brain volume during embryogenesis Reduced cerebellar volume	Reduced brain volume at age 4 months Reduced cerebellar volume	Reduced cerebellar volume	Hydrocephalus (6.5%)	Not reported
Cardiac defects	Septal defects similar, but not identical, to human DS	Not reported	None	Mainly VSD; also outflow tract defects and AVSD similar to human DS	Cardiac defects include ASD VSD and AVSD (in several models: Dp(16)1Yeh/+, Dp(16)2Yeh/+, Dp(16)4Yeh/+ and Dp(16)1Yeh/+; Dp (16)2Yeh/+; Dp(16)3Yeh/+	Not reported
Gastro-intestinal defects	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Thyroid disease	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Hematopoietic	MPD in adults No leukemia Macrocytosis Reduced adult HSC Impaired HSC self-renewal in adults	No MPD or leukemia Macrocytic anemia Normal HSC numbers and function in adults Impaired fetal liver HSC and progenitor function	Co-operates with GATA1s and MPL to induce AMKL Thrombocytosis, increased MKs and mild anemia in adults	No MPD or leukemia even with GATA1s Macrocytosis Increased MKs and erythrocytosis in older adults	Not reported	Not reported
	Increased GMP in adults	Increased GMP in adults	Increased GMP in adults			

AMKL, acute megakaryocytic leukemia; ASD, atrioseptal defect; AVSD, atrioventricular septal defect; DS, Down syndrome; GMP, granulocyte-macrophage progenitor; HSC, hematopoietic stem cell; MPD, myeloproliferative disorder; VSD, ventriculoseptal defect. See text for details.

neonates had trilineage perturbation of hematopoiesis with increased erythroid and myeloid cells and abnormal platelet development consistent with the effects of trisomy 21 on HSPC function persisting after birth [11]. In contrast, the few studies in adults with DS suggest that trisomy 21 causes a different profile of hematologic abnormalities later in life. Adults with DS have a high prevalence of red cell macrocytosis and quantitative and qualitative B- and T-lymphocyte abnormalities, while some have unexplained thrombocytopenia and neutropenia [77–79], myelodysplasia or bone marrow failure [80]. This suggests that in adults, trisomy 21 may induce HSC aging, consistent with recent studies in Ts65Dn mice implicating increased expression *USP16* as a possible mechanism for these effects [20].

Second, the mechanisms linking trisomy 21-mediated perturbation of fetal liver hematopoiesis and the high frequency of *GATA1* mutations in DS neonates are still unclear. Trisomy 21-mediated proliferation of fetal liver megakaryocyte/erythroid-biased HSPC may simply provide a permissive cellular environment for expansion of preleukemic mutant *GATA1* clones. Alternatively, changes to pathways regulating fetal HSPC growth and differentiation in DS may be directly responsible for the increased frequency of *GATA1* mutations. Similarly, the link between impaired B-cell development in DS fetal liver HSPC and the increase in B-ALL [69,81,82] and of immune deficiency in children with DS [83] is unclear, although delayed expression of the normal fetal B-cell development program might increase the likelihood of acquiring leukemogenic mutations in lymphoid genes in early childhood.

Third, given that alterations in the microenvironment can promote myeloproliferative disorders and leukemia in mouse models [84–87], the DS fetal liver microenvironment may support, or even drive, the abnormal growth and differentiation of DS fetal liver HSPC. The natural history of TAM, which resolves within a few weeks of life in most cases and is characterized by infiltration of the liver by mutant *GATA1* blast cells [88,89], also suggests that factors produced in the fetal liver microenvironment may be necessary to maintain these cells. In support of this, *in vitro* survival of TAM blast cells and *in vivo* survival of leukemia cells in a mouse model of DS-like acute myeloid leukemia has been shown to be dependent on insulin-like growth factors [90].

Finally, the molecular basis for perturbation of fetal liver HSPC growth and differentiation by trisomy 21 remains to be explained. Even using highly purified fetal liver HSPC, we found no significant increase in expression of selected trisomic genes on Hsa21 (*RUNX1*, *ERG*, *DYRK1A*) known to influence HSPC behavior and development of leukemia through gene dosage [51,91]. This does not exclude a role for trisomy 21 dose-related changes in these genes given limitations in the sensitivity of the methodology [92] and the confounding influence of interindividual variation [93] as discussed below, especially since even small changes in expression of the Hsa21 genes are associated with DS-like defects in mouse models [94] and multiple genes may be involved [95].

Animal models of leukemia and abnormal hematopoiesis in DS

Although ML-DS and TAM provide a natural human model to interrogate the impact of trisomy 21 on HSPC and the mechanisms which contribute to the development of leukemia in DS, mechanistic experiments to identify the exact role of specific genes are often difficult in human cells. Initial attempts to model ML-DS and TAM

in mouse models were disappointing as no spontaneous leukemias developed (Table 2). However, this is consistent with human DS where trisomy 21 dysregulates HSPC proliferation and differentiation but is insufficient to promote leukemia without additional, acquired mutations. All of the DS mouse models have abnormal hematopoiesis, typically affecting erythroid and megakaryocyte development [48–50], although the defects do not accurately recapitulate those seen in human fetal liver [45]. Nevertheless, by co-expressing additional oncogenes, DS mouse models provide potential insight into genes and pathways, which may contribute to perturbation of HSPC development by trisomy 21, including *ERG*, *DYRK1A*, *HMGNI* and *miR125b* [51,91,96–98].

The myeloproliferative disorder in adult Ts65Dn mice [48], for example, is clearly linked to gene dosage of *ERG* since reducing the number of copies of *ERG* from 3 to 2 in this model corrects the hematologic abnormalities [91]. Since neonatal TsDn mice are not affected, Birger *et al* [97] used a different approach to modeling TAM. Building on data showing potent effects of *ERG* overexpression on megakaryocyte proliferation and leukemia in disomic mice [100], they recently created a double transgenic mouse model of TAM/ML-DS on a non-trisomic background in which overexpression of *ERG* promoted fetal liver MEP expansion similar to that seen in human fetal liver, and this synergized *in vivo* with expression of *GATA1s* to cause a TAM-like disease and subsequent progression to megakaryocyte–erythroid leukemia [97]. Nevertheless, *ERG* has not yet been shown to be significantly overexpressed in trisomy 21-containing human hematopoietic cells, including leukemias [45,100] and hESC/iPSC [46,47].

Malinge *et al* [51] recently used Ts1Rhr mice, trisomic for 33 Hsa21 orthologs (Table 2), to create a trisomy 21-dependent ML-DS model by crossing them with *GATA1s* knock-in mice and overexpressing a transforming *MPL* allele (*MPL*^{W515L}), which has been reported in ML-DS [101,102]. In this model, they showed that *DYRK1A* was able to act as a megakaryoblastic tumor-promoting gene and they found increased expression of *DYRK1A* in human ML-DS samples [51]. Although this identifies a possible role for increased expression of *DYRK1A* in the transformation of TAM to ML-DS, this model does not fully recapitulate the human disease. For reasons that are still not clear, this model can only be produced in adult, and not fetal, hematopoietic cells, and indeed, *DYRK1A* expression does not appear to be significantly increased in human fetal HSPC [45], perhaps indicating altered mechanisms of *DYRK1A* regulation in fetal cells compared to postnatal or leukemic cells. Furthermore, *MPL*^{W515L} is able to induce a fatal, rapid onset myeloproliferative disorder even in the absence of *Gata1s* and a trisomic background [103] highlighting the importance of the cellular context in understanding the contribution of individual genes.

The Ts1Rhr mouse model has also proved useful for investigating the role of Hsa21 orthologs in B-cell development and B-ALL. Lane *et al* [98] recently showed that, as in human fetal liver [45], B progenitors were reduced in bone marrow from young Ts1Rhr mice but were more clonogenic than wild-type progenitors and could be replated indefinitely *in vitro*. Furthermore, Ts1Rhr B progenitors were transformed into B-ALL *in vivo* by *CRLF2* with activated *JAK2*, a known oncogenic stimulus in DS-ALL. Lane *et al* then identified differential expression of *PRC2* targets and sites of H3 K27 trimethylation as a specific ‘signature’ common to DS-ALL and

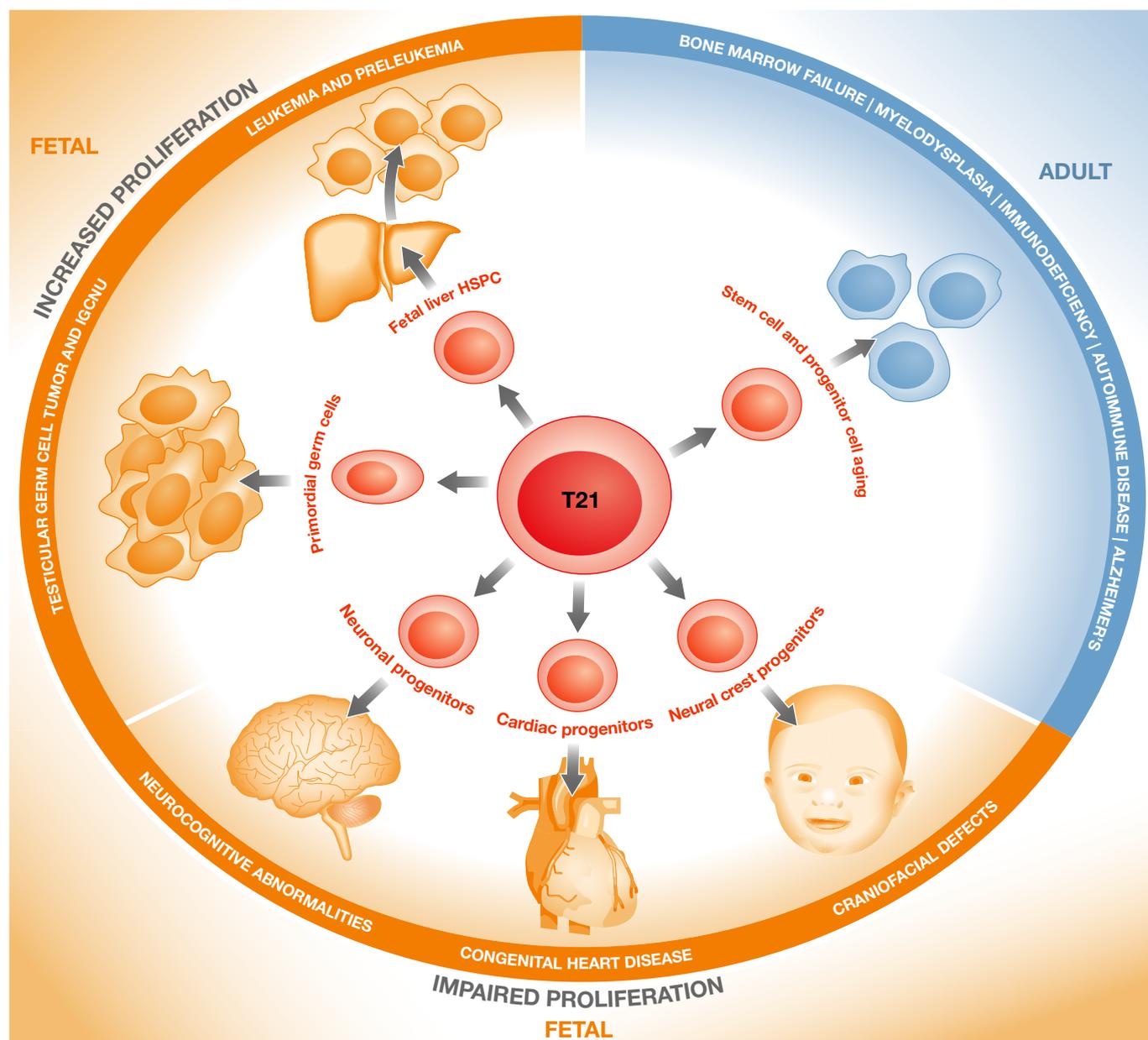


Figure 1. Impact of trisomy 21 on stem and progenitor cell function.

Studies in human cells and in animal models of Down syndrome (DS) show that trisomy 21 can affect the self-renewal, proliferation and differentiation of stem and progenitor cells either directly or via the supportive microenvironment. In fetal life in DS, proliferation of hematopoietic and testicular stem/progenitor cells is increased and susceptibility to malignant transformation (leukemia and testicular cancer) is increased in childhood. By contrast, proliferation of stem/progenitor cells of other lineages is impaired and is responsible for many of the developmental defects affecting the brain, craniofacial structures and heart in DS. After birth, trisomy 21 has been shown to cause premature aging of stem and progenitor cells both of hematopoietic and non-hematopoietic lineages, an effect which is likely to contribute to the phenotypic abnormalities in adults with DS, including Alzheimer's disease, bone marrow failure and impaired immunity.

Ts1Rhr B cells and, through a series of elegant experiments, showed that overexpression of *HMGN1*, an Hsa21 ortholog trisomic in Ts1Rhr mice which encodes a nucleosome remodeling protein, is responsible both for this gene expression signature and for the proliferative and leukemia-promoting effects on Ts1Rhr B cells. These data provide compelling evidence in support of a role for *HMGN1* in the perturbation of B-cell development by trisomy 21 and the increased susceptibility of children with DS to B-ALL.

USP16 and defects in HSC self-renewal and stem cell aging By comparing hematopoiesis in Ts65Dn, Ts1Cje and wild-type mice, Adorno *et al* [20] identified a role for the mouse homolog of the Hsa21 gene *USP16* in HSC self-renewal. HSC frequency was reduced by greater than threefold in Ts65Dn mice, which are trisomic for *USP16*, compared to Ts1Cje mice and wild-type mice, which have only 2 copies of *USP16*. HSC function was also impaired in the *USP16* trisomic mice with reduced clonogenicity and multilineage

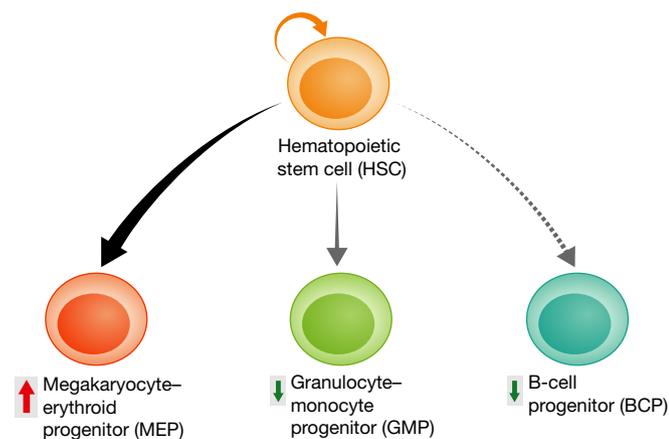


Figure 2. Perturbation of human fetal hematopoiesis by trisomy 21.

Comparison of the frequency and function of disomic and trisomy 21 second-trimester human fetal hematopoietic stem cells and progenitor cells (HSPC) has shown a consistent pattern of abnormalities in the trisomic populations. Trisomy 21 alters the balance of HSPC differentiation, promoting expansion and proliferation of megakaryocyte–erythroid progenitors (MEP) and megakaryocytes during the second trimester at the expense of both granulocyte–monocyte progenitors (GMP) and B-cell progenitors (BCP), which are both significantly reduced.

engraftment following secondary transplantation. These features were associated with a 1.5-fold increase in *USP16* gene expression and were reversed by short interfering RNAs. Interestingly, similar defects were seen in Ts65Dn neural progenitors and fibroblasts consistent with previously reported defective proliferation of primary human DS fibroblasts [104,105]. They also went on to demonstrate a link between trisomy for *USP16* and reduced activity of the PRC1 complex and its target *CDK2NA*, which regulate senescence and self-renewal of several somatic stem cell types [106,107; reviewed in 108]. The reduction in HSC frequency and clonogenicity contrasts with the increase in HSC frequency and clonogenicity in human DS fetal liver [45]. However, although this may reflect species-specific differences in hematopoiesis and/or the role played by other genes/pathways in the senescence of Ts65Dn mouse HSC, another important issue is age. The impaired HSC self-renewal reported by Adorno *et al* in adult Ts65Dn mice [20] is compatible with the increasing recognition of the occurrence of hematologic abnormalities, including myelodysplasia and bone marrow failure, in older adults with DS [80].

Non-hematologic cancers

It is likely that several mechanisms contribute to the 50% reduction in the frequency of solid tumors with DS, including the effects of trisomy 21 on stem and progenitor proliferation, tumor-associated angiogenesis and tumor suppression [reviewed in 22]. It is notable that the only malignancy, apart from leukemia, to be increased in DS is testicular germ cell tumors. These tumors are derived from primordial germ cells and, in DS, are believed to arise *in utero* through a pre-invasive stage known as intratubular germ cell neoplasia unclassified (IGCNU), which has been documented in the second trimester [109,110] and which is preceded by activation of signaling pathways leading to increased proliferation and impaired differentiation [60]. These cellular abnormalities are similar to those

in fetal HSPC, and, interestingly, testicular germ cell tumors also share several key signaling pathways, such as *KIT/SCF*, *K-RAS* and *PZLF* with normal and leukemic HSPC [60] and suggesting that increased susceptibility to both these malignancies in DS may derive from the trisomy 21-mediated proliferative drive to stem cells within fetal hematopoietic and testicular tissues.

Investigations into the mechanisms of reduced tumor susceptibility in DS, largely through studies in mouse models, suggest that 3 Hsa21 genes, *ETS2*, *RCAN1* and *DYRK1A*, may play a role. Through crossing Ts1Rhr mice with *Apc^{Min}* mice, which are heterozygous for the adenomatosis polyposis coli gene, and *Ets2^{+/-}* mice, Sussan *et al* [111] showed that protection against colonic tumors in this model was in part dependent on the presence of three copies of *ETS2*. This suggests that *ETS2* can act as a tumor suppressor, through as yet unclear mechanisms, but that other genes may contribute to this effect. Subsequently, Baek *et al* [112] used the Ts65Dn mouse model and a transgenic disomic mouse over-expressing *RCAN1* to show that increased expression of *RCAN1* was sufficient to suppress growth of lung cancer and melanoma cell lines *in vivo* through inhibition of VEGF-mediated tumor angiogenesis by suppressing the calcineurin pathway in co-operation with *DYRK1A*. However, more recent experiments in a more aggressive tumor model (NPcis) found that trisomy improved survival rather than preventing cancer and that neither *ETS2* nor tumor angiogenesis was responsible for this protective effect [113]. Taken together, these studies indicate that the mechanisms underlying tumorigenesis in DS result from a complex interplay between changes in expression of Hsa21 and other genes, inter-individual differences in genetic susceptibility and acquired changes in the microenvironment.

Craniofacial defects

In contrast to the hematopoietic system, there is limited information about the effects of trisomy 21 on the stem and progenitor cells involved in craniofacial development during fetal life, especially in humans. Most of the insight into the mechanisms by which trisomy 21 causes the craniofacial defects in DS has relied on animal models. Using detailed imaging, Richtsmeier *et al* [114] have shown that the characteristic craniofacial defects in individuals with DS are closely mimicked early in development in Ts65Dn mice and, to a slightly lesser extent, in Ts1Cje mice [115]. In particular, there is hypoplasia of the mandible and mid-facial skeleton [114], structures which in normal mouse development are known to be derived from cells which migrate from the cranial neural crest to populate the craniofacial precursors of the mid and lower face [reviewed in 116]. Using Ts65Dn mice crossed to mice expressing lacZ under the control of the *Wnt1* promoter, Roper *et al* have made a number of important observations about mechanism of these DS-associated craniofacial abnormalities. First, they demonstrated that the number of neural crest cells was significantly reduced in trisomic compared to control (euploid) embryos [117]. Second, they showed that this was due both to reduced generation of neural crest cells and to impaired migration into the first pharyngeal arch (PA1), which goes on to form the maxilla and lower jaw. They then found that *in vitro* proliferation of these trisomic PA1-derived cells in short-term culture was reduced compared to euploid controls. The defect in proliferation of the PA1 cells was partially rescued by addition of the mitogen Sonic Hedgehog (Shh), suggesting that the

defects in neural crest generation and proliferation might be due, at least in part, to impaired Shh responsiveness [117]. This is interesting because, as discussed below, abnormal Shh signaling is also implicated in the reduced proliferation of cerebellar granule precursors in the Ts65Dn mouse, although whether this is directly or indirectly linked to a specific trisomic gene(s) is not yet clear [118,119].

To identify genes and pathways which underlie the defects in PA1 neural crest cells, Billingsley *et al* [120] isolated mandibular precursor cells from embryonic day 13.5 (E13.5) Ts65Dn mice and compared their gene expression with the same cell population isolated from euploid controls using microarray. Of the relatively small number of differentially expressed genes, 20 contained homeobox DNA-binding domains, including increased expression of at least two genes (*EN2* and *OTX2*) reported to have a role in mandibular development [121,122], reduced expression of all 12 of the differentially expressed HOX genes and a modest increase (1.2-fold) in expression of *SOX9*, known to be important for normal skeletal development [120]. The extent to which these changes in gene expression are linked to the craniofacial defects in Ts65Dn mice and how they are linked to trisomy is an intriguing puzzle which remains to be investigated particularly given that expression of Ts65Dn trisomic genes in the mandibular precursor cells was not increased compared to euploid controls.

The most studied candidate genes on Hsa21 linked to the craniofacial abnormalities in DS are *DYRK1A*, *RCAN1* (*DSCR1*) and *ETS2* [94,123–125]. Arron *et al* noted the similarity between the craniofacial defects in calcineurin-deficient and Nfatc-deficient mice and those seen in DS and went on to show that *DYRK1A* and *RCAN1* can act synergistically to prevent activation of NFATc-target genes and would therefore be plausible mediators of the craniofacial defects in DS. However, the role of *DYRK1A* and *RCAN1* in craniofacial development was not directly addressed in this study, and therefore, the extent to which perturbed NFATc-signaling due to increased *DYRK1A/RCAN1* expression contributes to craniofacial defects in DS remains unclear [94]. Studies in DS mouse models have more directly addressed the role of *DYRK1A*, *RCAN1* and of *ETS2* in the craniofacial defects [112,123,126–128]. Taken together, these studies suggest that trisomy of each of these genes individually is insufficient to cause the characteristic DS-associated craniofacial phenotype.

It is clear that interpreting the impact on craniofacial development of differences in expression of individual genes in DS mouse models is extremely difficult and needs to take into account differing mouse genetic backgrounds, as well as developmental stage, cellular context and interactions between other trisomic and non-trisomic genes [23]. As in primary human fetal hematopoietic cells, perturbation of craniofacial development by trisomy 21 may be largely mediated via non-trisomic genes and/or by small changes in the level of expression of multiple trisomic genes which are difficult to detect using standard methods. Nevertheless, the close match between the Ts65Dn mouse and human phenotype, the ability to alter copy number of individual genes or groups of genes in this model and the identification of the relevant stem/progenitor cells now provide crucial tools to investigate candidate genes in DS craniofacial defects and the mechanisms by which they are linked to trisomy 21.

Abnormalities of brain structure and function

Studies in individuals with DS and in DS mouse models indicate that intellectual disability in DS is directly related to impaired development

of many areas of the brain, including the cerebellum, the visual, auditory and somatosensory cortex, the motor cortex and the superior temporal gyrus [129]. Many of the available DS mouse models recapitulate the structural and functional brain abnormalities of human DS very closely [reviewed in 24,32,39,130]. Here, we briefly discuss recent studies which have investigated the cellular and genomic basis for these defects.

The most consistent finding, both in DS animal models and in primary human samples, is of reduced cell numbers in several specific areas of the brain [119,126,129–136]. In second-trimester human DS fetal brain, a number of studies have shown that total cell numbers are reduced in the hippocampus, dentate gyrus, parahippocampal gyrus [131,132] and cerebellum [133]. Importantly, there is a particular reduction in neuronal precursor cells while astrocytic cells are preserved [132]. Assessment of the proliferative status of these cells using immunohistochemical staining for the cell cycle-associated marker Ki-67 suggests that there are fewer proliferating cells in these regions of the brain in DS samples [131–133] compared to controls together with a higher frequency of apoptotic cell death in some areas [132]. Detailed functional studies and characterization of the stem and progenitor cells populations have not yet proved possible in these tissues. However, the findings suggest that trisomy 21 causes impaired neurogenesis in DS from early in fetal development and may also affect cell fate specification (from neurones to astrocytes).

Studies in DS mouse models support the observations in human brain. Several groups have shown that neurogenesis is impaired in several areas of the brain including the hippocampus, neocortex, dentate gyrus and cerebellum and that many of these changes begin during fetal or early postnatal development [118,119,129,134–136]. Histological studies in Ts65Dn mice indicate that there are reduced numbers of mitotic cells compared to euploid mice [119,135], and more recently, administration of BrdU confirms reduced proliferation of cells in the same areas of the brain [137]. There is good evidence, both from *in vivo* studies and *in vitro* culture of neural precursor cells [118,129], of altered cell fate specification as a result of which, as in human fetal brain, the reduction in neurogenesis is accompanied by an increase in astroglialogenesis [127,131–133,136–138].

Insight into mechanisms of impaired neurogenesis has come mainly from investigation of pathways known to be important for normal neurogenesis, such as Shh, and from specific investigation of candidate genes on Hsa21, including *DYRK1A*, *RCAN1*, *GRIK2* and *APP* [118,139,140]. In particular, recent studies report dramatic improvements in neurogenesis in response to pharmacological agents, thereby implicating defects in the pathways they target in the pathogenesis of the cognitive defects in DS [141–143].

Several lines of evidence link abnormalities in the Shh pathway to the defects in neurogenesis in DS. First, cerebellar granule cell precursors isolated from the Ts65Dn DS mouse model have reduced *in vitro* responsiveness to Shh [119], which is known to be a potent mitogen for normal granule cell precursors [144]. Second, administration of a Shh agonist (SAG-1) to neonatal Ts65Dn mice restores cerebellar development to normal in adult mice and improves learning and memory [141], supporting a significant role for the Shh pathway in the pathogenesis of DS-associated cognitive defects. These data are particularly interesting given the putative role of Shh in the craniofacial defects

in DS [117,118]. Although no direct link between Shh and trisomy 21 was established in those studies, clues to the role of trisomy 21 may lie with studies into the role of the Hsa21 gene *APP*. Triplication of *APP* in Ts65Dn impairs neuron precursor proliferation, differentiation and maturation [118,129]. These effects are dependent upon the APP intracellular domain (AICD), and increased levels of AICD lead to increased transcription of the *Ptch* gene, leading to dysregulation of the Shh pathway [118]. AICD may also be involved in another pathway important in the impaired neurogenesis in Ts65Dn mice by directly interacting with, and increasing the activity of, glycogen synthase kinase 3b (GSK3 β), a key negative regulator of neuron proliferation, differentiation, maturation and migration [145]. Trazzi *et al* [118] recently showed that treatment of Ts65Dn mice with lithium, a GSK3 β inhibitor, normalized neural precursor proliferation, cell fate specification and maturation, suggesting that dysregulation of the GSK3 β signaling pathway, potentially by the AICD of APP, also plays a significant role in the impaired neurogenesis typical of DS. Interestingly, fluoxetine, a 5-HT1A receptor agonist, has also recently been shown to improve neurogenesis in the Ts65Dn mouse model. Fluoxetine increased total and proliferating neural progenitor cells, corrected defective 5-HT1A receptor expression and rescued defects in contextual memory and behavior typical of DS both in fetal [142,143] and adult Ts65Dn mice [137,143,146]. These responses may be due to direct effects of fluoxetine on the serotonergic system. However, as Trazzi *et al* [143] showed that activation of 5-HT1A receptors by fluoxetine inhibits GSK3 β , this would provide a mechanistic link to Hsa21 (inhibition of the *APP*-driven increase in GSK3 β activity) for the beneficial effects of fluoxetine on neurogenesis, behavior and memory in DS.

Comparison of the defects in Ts65Dn and Ts1Rhr mice (Table 2), which are trisomic for only 33 Hsa21 orthologs, with a mouse model monosomic for these genes (Ms1Rhr) identified *DYRK1A*, *GIRK2* and *SIM2* as necessary, but not sufficient, for hippocampal-based learning deficits in Ts65Dn [140]. Several lines of evidence support a role for increased expression of *DYRK1A* in DS-associated cognitive defects [125,139,147–151]. In disomic transgenic mice, overexpressing *DYRK1A* by 1.5–2-fold in cortical neurons leads to impaired neural progenitor differentiation and motor and cognitive defects [125] which are ameliorated by selective *DYRK1A* knockdown [151]. More recently, Altafaj *et al* [139] showed that *in vivo* knockdown of *DYRK1a* to normal levels in trisomic mice (Ts65Dn) by shRNA also rescues functional and behavioral defects in these mice consistent with a role for increased *DYRK1A* in their pathogenesis. These data are supported by Hibaoui *et al* [152] who reported that impaired neural differentiation of trisomy 21 iPSC was rescued by a selective *DYRK1A* inhibitor. The exact mechanism(s) by which *DYRK1A* affects neurogenesis and neuronal differentiation is not yet clear but includes *DYRK1A*-mediated de-regulation of the master regulator of neuronal differentiation NRSF/REST [153] and inhibition of choline acetyltransferase induction [154].

Cardiac abnormalities

Both Ts65Dn and Tc1 mice (Table 2) exhibit heart defects similar to those observed in DS, suggesting that trisomy of one or more of the ~100 genes common to these models may be responsible for the cardiac defects in DS [30,155,156]. Indeed, a recent study

has reported that the smallest critical region in a mouse model (Dp(16)4Yeh/+) associated with cardiac defects, including atrial and ventricular septal defects, can be reduced to a 3.7-Mb region containing 35 genes [157]. Although elegant experiments in transgenic mice provide a guide to the regions of Hsa21 likely to be critical for cardiac defects in individuals with DS, the frequency of these defects in DS mouse models [30,156] is considerably lower (5–15%) than in humans (40–50%; Table 1), suggesting a very complex etiology involving multiple Hsa21 and non-Hsa21 genes.

In comparison with studies in hematopoietic, craniofacial and brain tissue, little is known about the cellular and molecular basis for these defects in cardiac development. Using BrdU labeling, Fuchs *et al* [158] found that neonatal Ts65Dn mice had fewer proliferating cells in the left and right heart walls and septum compared to euploid mice. Interestingly, in the same experiments, they also found reduced numbers of BrdU-positive cells in the intestine, liver and skin in Ts65Dn mice compared to euploid mice, supporting the contention that trisomy 21 impairs the proliferation of progenitor cells of a wide variety of non-hematopoietic tissues during development. The mechanism(s) is not yet clear. Limited gene expression studies of whole human fetal cardiac tissue suggest that dose-dependent upregulation of Hsa21 genes might alter the expression of mitochondrial function genes although this does not explain why cardiac defects affect only half of DS individuals [159]. However, progress in understanding the genetic basis of congenital heart defects, such as AVSD, in the absence of trisomy 21, provides some clues to the potential pathways in DS, including *HOX* genes [160,161], the Shh pathway [162,163], the VEGF pathway [164] and a number of chromatin remodeling genes, including *MLL2* and *CHD7* [reviewed in 165]. Interestingly, Ackerman *et al* [166] recently used a candidate gene resequencing approach to identify potentially damaging variants in six genes at approximately sevenfold higher frequency in DS individuals with AVSD, including 2 Hsa21 genes (*COL6A1*, *COL6A2*) and one gene involved in Wnt signaling (*FRZB*); all six genes identified in this study are implicated in VEGF-A signaling known to be important for normal heart septation [164]. The recent refinements in techniques to generate cardiomyocytes and cardiac progenitors *in vitro* from ESC and iPSC [167] have provided insight into developmental heart defects [168] and may also prove a useful approach for further investigating the mechanisms of defects in cardiac development in DS at the cellular and molecular level [169].

Genomic basis for phenotypic variation in DS

Current estimates identify 243 protein-coding genes on Hsa21 as well as 259 long non-coding RNAs and 138 short non-coding RNAs [5]. There is general consensus that changes in the pattern and level of expression of one or more Hsa21 genes are responsible, directly or indirectly, for the abnormalities in stem/progenitor cell function and, ultimately, for the clinical features of DS. The conventional view is that most of these features occur due to imbalanced dosage of Hsa21 genes. However, increasing evidence indicates that the genetic landscape is far more complex than can be accounted for by a simple dosage effect and that trisomy 21 exerts its effects in stem and progenitor cells in several ways.

First, for some Hsa21 genes (probably a minority), mouse models show that in some tissues, increased expression of a single gene is sufficient to produce changes in stem/progenitor cell behavior which correlate with clinical phenotypic read outs, such as leukemia (*ERG*, *DYRK1A*, *HMGN1* or *miR125b*) or cognitive defects (*APP*) [51,91,96–98,118,129]. Conclusive evidence for a simple dosage effect of individual Hsa21 genes in human stem/progenitor cells is lacking at present. A more likely scenario is that several Hsa21 genes, either acting in a common pathway (e.g., NFAT or Wnt signaling) or independently, cause the phenotypic effects in DS [94,95,170]. Interestingly, Emmrich *et al* [95] demonstrated one mechanism by which increased expression of a group of Hsa21 genes might affect several target genes and alter HSPC behavior. They showed that coordinated expression of 3 Hsa21 miRs as a miR99a/let7c/miR125b tricistron by lentiviral transduction of cord blood CD34⁺ HSPC caused expansion of megakaryocyte progenitors and modulation of target genes in the TGF β and Wnt signaling pathways.

In human tissues, microarray has been used to identify differential expression patterns in trisomic versus euploid cells. Interestingly, in almost all gene expression datasets, only a small minority of the 243 Hsa21 protein-coding genes are significantly differentially expressed (Fig 3; Supplementary Tables S1 and S2). The low number of differentially expressed Hsa21 genes may be due to limited sensitivity of microarray to detect the small changes expression (1.2- to 1.8-fold) expected in trisomic cells [discussed in 92]. Such changes might also be masked by inter-individual variation in expression of Hsa21 genes, as seen for non-Hsa21 genes [93]. Nevertheless, in principle, small changes in expression of multiple Hsa21 genes, each of which is not identified as statistically significant, may still cause critical dysregulation of stem/progenitor cell function. Figure 4 illustrates simple scenarios by which trisomy 21 may lead to a range of possible effects on gene expression and protein production (even without taking into account the impact of epigenetic mechanisms and interindividual variation). Mathematical modeling, supported by experimental evidence [171,172], shows that three dosage effects on gene expression are commonly found in aneuploids: a direct transacting effect, an inverse transacting effect and gene dosage compensation [171,173]. While the direct effect would result in a 1.5-fold increase in expression of genes on the trisomic chromosome, inverse transacting effects (where a gene on the trisomic chromosome regulates a gene on another chromosome) account for otherwise unexpected reductions in gene expression below the normal euploid level. On the other hand, dosage compensation arises when direct and inverse effects are counter-balanced, for example, if a gene on the trisomic chromosome regulates another gene on the same chromosome. Thus, dosage compensation ('buffering') may explain, at least in part, the small number of significantly differentially expressed genes in mouse and human trisomy 21 datasets.

Second, gene expression studies in human DS tissues (Supplementary Tables S1 and S2) [61,71,72,90,93,95,100,102,152,159,174–191], as well as DS mouse models, show extensive dysregulation of non-trisomic (euploid) genes as well as trisomic genes in DS (Fig 3), which are linked to DS-specific phenotypes in DS mouse models [35,93,192]. In a recent fascinating study in fibroblasts and iPSC from a unique set of monozygotic twins where one twin had trisomy 21 and the other did not (as a result of abnormal chromosome segregation prior to twinning), Letourneau *et al* [93] reported

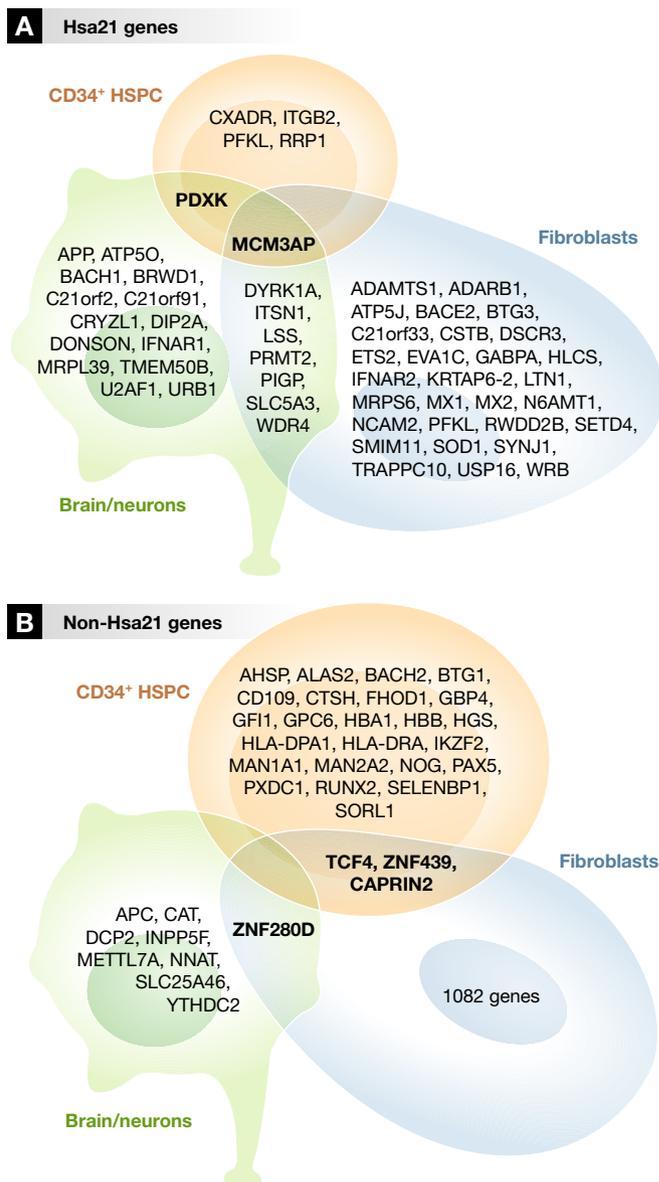


Figure 3. Differential expression of Hsa21 genes and non-Hsa21 genes in hematopoietic and non-hematopoietic cells.

Human Gene Expression Profiling (GEP) datasets from public repositories were extracted, and differential expression analysis was performed using the limma algorithm (see Supplementary Tables S1 and S2). The data analyzed here are from references [187,189,190]. In order to compare gene expression by these non-hematopoietic cells with non-malignant hematopoietic cells, we used our own unpublished gene expression data from human trisomy 21 ($n = 4$) and normal ($n = 3$) second-trimester fetal liver CD34⁺ HSPC samples obtained using the Affymetrix HuEX_1-0_st array. An FDR cutoff of 0.15 was used. There was almost no overlap between the differentially expressed genes in different tissue types: Only a single Hsa21 gene, *MCM3AP* (minichromosome maintenance complex component 3-associated protein) (A), and no non-Hsa21 genes were differentially expressed in both hematopoietic and non-hematopoietic cells (B).

changes in gene expression across every chromosome. Furthermore, they found a consistent pattern of alternating regions of increased and decreased gene expression across large chromosomal segments which they called 'gene expression dysregulation domains' (GEDDs). Remarkably, GEDDs with increased expression in trisomic cells

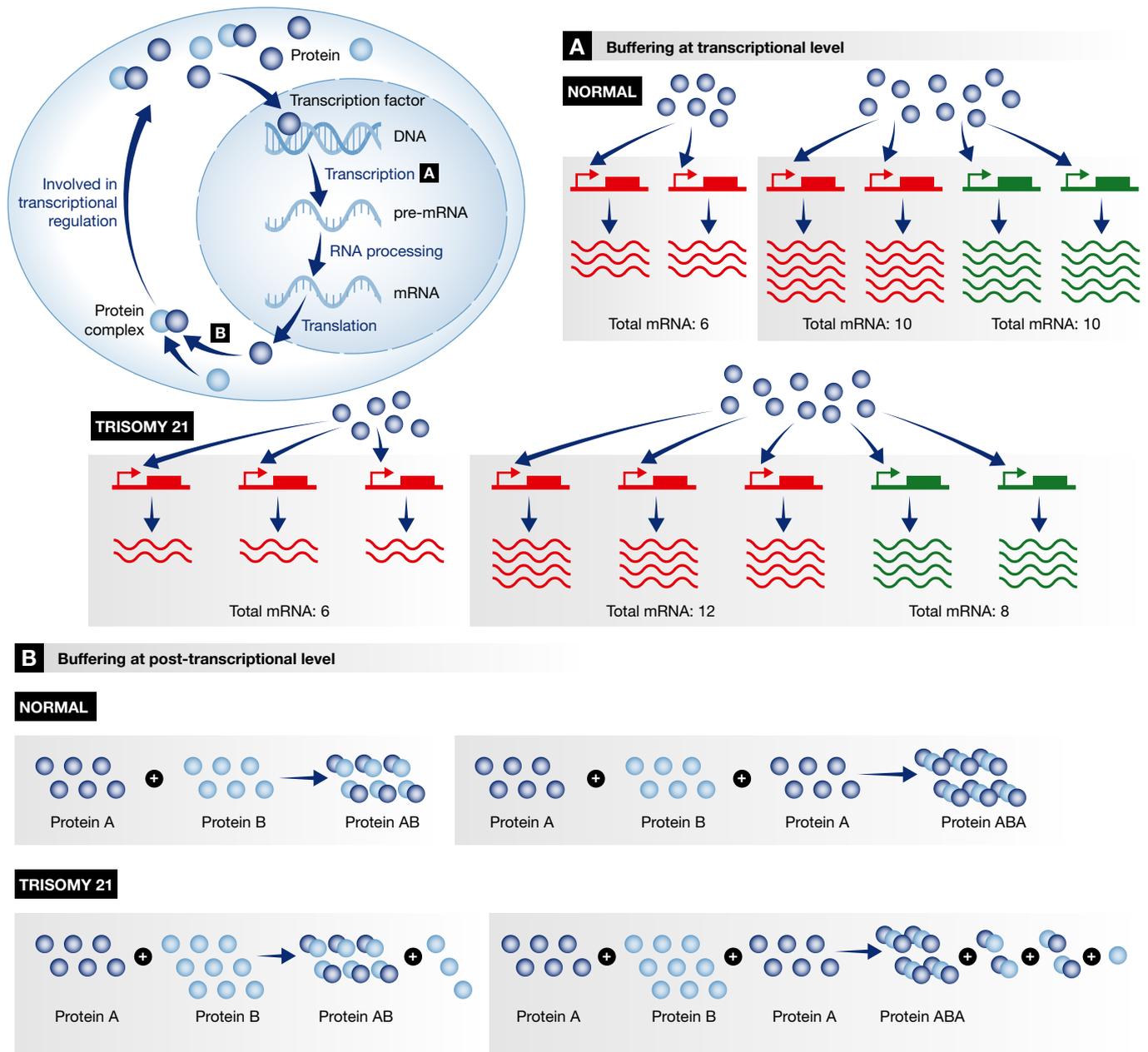


Figure 4. Models predicting the consequence of increased gene dosage due to trisomy in stem and progenitor cells.

This shows various scenarios by which increasing gene dosage to 150% (as in trisomy) may cause a range of effects at the transcriptional and post-transcriptional level which lead to stoichiometric imbalances which may 'buffer' the effect of dosage imbalance of trisomic genes. Trisomy 21 is used as an example. For simplicity, these models do not take into account the additional impact of epigenetic mechanisms and interindividual variation. (A) Effects at the transcriptional level. Left: A gene on Hsa21 (red) is regulated by a transcription factor (blue circle) present in a limited and similar amount in normal and trisomy 21 cells. In this case, transcription is the limiting factor: The additional gene will have no impact on the amount of mRNA produced, and the overall level of expression of the gene is the same in both trisomy 21 and normal (euploid) cells. Right: A gene on Hsa21 (red) is co-regulated by a gene on another chromosome (green) by a transcription factor (blue circle) which is present in limited amounts. The total quantity of mRNA produced is still constrained by the limited quantity of transcription factor which must now be shared between expression of five genes rather than four. The expression of the three copies of the (red) Hsa21 gene will now consume 3/5 of the transcription factor (compared to 2/4 in the euploid state); therefore a scaling of $(3/5) / (2/4) = 3 \times 4 / (2 \times 5) = 12/10 = 120\%$. The expression of the two copies of the (green) non-Hsa21 gene will, instead, consume 2/5 instead of 2/4; therefore a scaling of $(2/5) / (2/4) = 4 \times 2 / (5 \times 2) = 80\%$. Thus, in this case, the additional copy of Hsa21 causes only a small increase in expression of the Hsa21 gene (to 120%), and this is matched by a decrease in expression (to 80%) of the other, non-Hsa21 gene. (B) Effects at the post-transcriptional level. Buffering effects at the protein level are related to the formation of a complex of proteins. Two examples are illustrated here. Left: A complex AB is formed by a protein A (dark blue circle) and a protein B (light blue circle). If the amount of protein B is increased by a factor of 1.5, but the amount of protein A remains constant, the number of AB complexes will not be increased above the normal level. Right: A complex ABA is formed by a ratio of 2 monomers of protein A and one monomer of protein B through intermediate complexes (AB or BA). When the amount of protein A is exactly twice the amount of protein B, all the proteins are used to form the complexes ABA. However, a 1.5-fold increase in the amount of protein B may lead to a decrease of the amount of ABA complexes since the production of the intermediate AB and BA complexes cannot be completed due to an insufficient amount of A monomers. In addition, as illustrated in the top left of the figure, buffering effects at the protein level may also influence the level of gene expression if the protein complex is itself involved in transcriptional regulation.

corresponded to areas which were normally repressed, while GEDDs with decreased expression corresponded to areas where transcription would normally be active. The mechanism(s) by which the chromatin environment is altered in this way by trisomy 21 has not yet been identified. No comparable data exist for other human trisomies, and so, it is also possible that these effects are due to the physical presence of an additional chromosome in the nucleus rather than specific to Hsa21. It also remains to be seen whether such altered global gene expression patterns are also seen in stem and progenitor cells from other tissues (e.g., HSPC) in individuals with DS.

Since the effects of trisomy 21 on stem/progenitor cells vary depending on the cellular context and stage of development, it is likely that the gene expression patterns responsible for these effects will also vary in different cell types. To address whether there was any overlap in differentially expressed genes between different tissue types in DS, we performed differential expression analysis of non-hematopoietic microarray datasets (Supplementary Tables S1 and S2) with our own gene expression dataset of primary fetal liver CD34⁺ HSPC. We found almost no overlap between the differentially expressed genes in different tissue types (Fig 3). Indeed, no non-Hsa21 genes and only a single Hsa21 gene, *MCM3AP*, were differentially expressed in both hematopoietic and non-hematopoietic cells. Although *MCM3AP* may be of interest since it is essential for initiation of DNA replication and mutations in families with inherited intellectual disability have been reported [193], the impact of increased levels of expression on hematopoietic and non-hematopoietic cells is unknown.

Conclusion

There is increasing recognition that trisomy 21 impacts on stem and progenitor cell function in many different ways. These changes in stem/progenitor cell behavior reflect adaptive epigenetic, transcriptional and/or translational regulatory mechanisms which allow cells to survive and function despite the presence of an additional copy of an entire chromosome. The effects of trisomy 21 on stem and progenitor cells are cell context dependent and developmental stage specific (Fig 1). During fetal and embryonic life, proliferation of hematopoietic and testicular stem and progenitor cells is increased and coupled with altered differentiation, which may underlie the unique susceptibility of individuals with DS to tumors of these two cell types. By contrast, the effects of trisomy 21 on progenitor cells of other lineages (e.g., cardiac, neural and intestinal) during early development are manifested mainly as impaired, rather than enhanced, cell proliferation which may protect these cells from subsequent malignant transformation and explain the reduced frequency of non-hematopoietic cancers in DS. Finally, trisomy 21-mediated premature aging of stem/progenitor cells may contribute to the phenotypic abnormalities in many tissue types, particularly in adults with DS. Although changes in the pattern and level of expression of one or more of these genes on Hsa21 are likely to be responsible, for the abnormalities in stem/progenitor cell function and, ultimately, for the clinical features of DS, increasing evidence indicates that these effects are mediated by complex genetic and epigenetic mechanisms beyond increased Hsa21 gene dosage. Uncovering the molecular mechanisms underpinning these defects in stem and progenitor cell function remains an exciting

Sidebar A: In need of answers

- The molecular mechanism by which gene dosage is regulated in a developmental stage- and tissue-specific context in trisomic stem and progenitor cells
- Reasons for the variable penetrance of abnormal phenotypes seen in individuals with aneuploidy
- The role of non-coding RNAs in the abnormalities of hematopoietic and non-hematopoietic stem and progenitor cells in human trisomies
- Mechanisms by which epigenetic changes are driven by trisomic genes; either directly or via their effects on other (disomic) genes

challenge and is at last beginning to offer real prospects of translation of these findings into useful therapeutic advances for individuals with DS.

Supplementary information for this article is available online: <http://embor.embopress.org>

Acknowledgements

The authors would like to thank members of the Roberts and Karadimitris labs (especially Tassos Karadimitris, Centre for Haematology, Imperial College London, UK), Professor Michael Stumpf (Centre for Bioinformatics, Division of Molecular Biosciences, Imperial College London, UK) and Professor Paresch Vyas (University of Oxford, UK) and members of his laboratory (especially Kelly Perkins) for helpful discussions over many years. We would also like to thank Professor Vaskar Saha and his laboratory (University of Manchester, UK) and Dr. Ollie Tunstall for help with the microarray experiments in human fetal CD34⁺ cells. This work was supported by Leukaemia Lymphoma Research, Children with Cancer and the Medical Research Council.

Author contributions

BL, SF, AR and IR analyzed data and wrote the paper.

Conflict of interest

The authors declare that they have no conflict of interest.

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