

Trisomy 21 Driven Pro-Inflammatory Signalling in Fetal Bone Marrow May Play a Role in Perturbed B-Lymphopoiesis and Acute Lymphoblastic Leukemia of Down Syndrome

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Introduction: Children with Down syndrome (DS) have a markedly increased risk of acute lymphoblastic leukemia (ALL), suggesting that trisomy 21 (T21) has specific effects on hematopoietic stem and progenitor cell (HSPC) biology in early life. Data from human fetal liver (FL) indicates that T21 alters fetal hematopoiesis, causing multiple defects in lympho-myelopoiesis. The impact of T21 on fetal B lymphopoiesis and how this may underpin the increase in ALL is not well known. We have recently found that fetal bone marrow (FBM) rather than FL is the main site of B lymphopoiesis; with a marked enrichment of fetal-specific progenitors (early lymphoid progenitors, ELP and PreProB progenitors) that lie upstream of adult type ProB progenitors (O'Byrne *et al*, *Blood*, *in press*). Previous preliminary data suggested that B progenitors were also reduced in T21 FBM (Roy *et al*, *Blood*. 124, 4331).

Aim: To dissect putative molecular mechanisms responsible for the defects in T21 FBM B-lymphopoiesis and its association with childhood DS ALL.

Methods: Second trimester human FBM and paediatric ALL samples were obtained from the Human Developmental Biology Resource and UK Childhood Leukaemia Cell Bank respectively. Multiparameter flow cytometry/sorting, transcriptome analysis by RNA-sequencing and microarray, and stromal co-culture assays were used to characterize HSPC and mesenchymal stromal cells (MSC) from normal (NM) disomic (n=21-35) and T21 (n=7-12) human FBM; RNASeq was performed on cytogenetically matched non-DS (n=13) and DS ALL (n=7).

Results: In contrast to NM FBM, fetal specific progenitors were virtually absent (CD34+CD10-CD19-CD127+ ELP $2.8 \pm 0.4\%$ vs. $0.8 \pm 0.4\%$ of CD34+ cells) or very severely reduced (CD34+CD10-CD19+ PreProB 12.8 ± 1 vs $2.6 \pm 0.7\%$) in T21 FBM. This was despite a >4-fold increase in the frequency of immunophenotypic HSC ($4.2 \pm 1.2\%$ vs $0.9 \pm 0.2\%$ of CD34+ cells) and similar frequencies of MPP and LMPP in T21 FBM. As in adult BM, the vast majority of B progenitors in T21 FBM were CD34+CD10+CD19+ ProB progenitors with a frequency ($28.8 \pm 8.3\%$) similar to NM FBM ($30.3 \pm 2.3\%$ of CD34+ cells). Thus, T21 causes a severe block in B-progenitor commitment at the LMPP stage, in tandem with a compensatory expansion of ProB progenitors. Consistent with this, T21 FBM HSC, MPP and LMPP had reduced B cell potential *in vitro* compared to NM FBM in MS5 co-cultures. RNAseq of NM (n=3) and T21 (n=3) FBM HSPC demonstrated global transcriptomic disruption by T21, with increased gene expression in HSC, MPP, LMPP and ProB progenitors. Cell cycle genes were enriched in T21 ProB progenitors. Despite these functional and global gene expression differences, expression of key B-lineage commitment genes was maintained suggesting the defect in B-lymphopoiesis may be secondary to lineage skewing of multipotent progenitors towards a non-B lymphoid fate and/or mediated by extrinsic factors. GSEA pointed to a role for multiple inflammatory pathways in T21 hematopoiesis with dysregulation of IFN α , IL6 and TGF β signalling pathways in T21 HSC/LMPP.

To investigate the role of the T21 microenvironment, we co-cultured NM HSC, MPP and LMPP with T21 or NM primary FBM MSC. T21 FBM MSC (n=3) had reduced capacity to support B cell differentiation *in vitro* consistent with perturbation of MSC function by T21. Similar to T21 FBM HSPC, transcriptomic analysis of T21 FBM MSC by microarray showed enrichment for IFN α signalling compared to NM; and T21 HSPC and MSC both showed increased gene expression for IFN α receptors *IFNAR1* and *IFNAR2*, which are encoded on chromosome 21. Since IFN α was undetectable by ELISA of conditioned media from NM and T21 MSC, differences in secreted IFN α from MSC are unlikely to fully explain the increased IFN signalling in T21 HSPC and MSC. This suggests that T21 may drive autocrine rather than paracrine

IFN signalling in FBM cells. Finally, RNASeq showed perturbed inflammatory signalling in DS ALL compared to non-DS ALL, suggesting a role for T21-driven inflammatory pathways in the biology of DS ALL.

Conclusions: These data show that T21 severely impairs B lymphopoiesis in FBM and is associated with expression of proinflammatory gene expression programs in T21 FBM HSPC and MSC and DS ALL. The compensatory expansion of T21 FBM ProB progenitors, through self-renewal or via an alternative differentiation pathway; with concomitant T21-driven proinflammatory signalling may underpin the increased risk of B progenitor ALL in childhood.

Disclosures

No relevant conflicts of interest to declare.

Author notes

*Asterisk with author names denotes non-ASH members.