

Abstract Submission

23. Hematopoiesis, stem cells and microenvironment

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GENOME WIDE DYSREGULATION OF GENE EXPRESSION BY TRISOMY 21 IN FETAL LIVER HAEMATOPOIETIC STEM AND PROGENITOR CELLS

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Background: Trisomy 21 (T21) causes multilineage perturbation of fetal and neonatal haematopoiesis. We have previously demonstrated that 2nd trimester Down Syndrome (DS) fetal liver (FL) samples have increased numbers of immunophenotypic haematopoietic stem cells (HSC) and megakaryocyte erythroid progenitors (MEP) together with a severe reduction in committed B progenitors (CBP). Functional studies and Fluidigm gene expression assays support lineage bias favouring megakaryocyte-erythroid over B lymphoid differentiation and proliferation but did not identify causative genes on chromosome 21 (Hsa21) reflecting the limited number of Hsa21 genes which can be included on the panel¹. Determining the mechanisms underlying these defects is likely to be important for understanding why young children with DS are more prone to myeloid and lymphoid leukaemias.

Aims: To investigate the impact of the additional copy of Hsa21 on the transcriptome of primary FL HSC and progenitor cells (HSPC). Specifically, to determine (a) whether there is a T21 'gene expression signature' and whether this is consistent across all HSPC types and samples and (b) whether T21 causes consistent patterns of genome-wide dysregulation of gene expression in HSPC.

Methods: Genome-wide transcriptome profiling was performed by RNA sequencing of 8 flow-sorted FL HSPC populations (immunophenotypic HSC, MPP, LMPP, CMP, MEP, GMP, ELP and CBP as previously described¹) from 7 gestation-matched 2nd trimester FL samples: DS (n=4) and normal (n=3). Indexed cDNA libraries were multiplexed and sequenced using Illumina HiSeq2500. Raw reads generated were subjected to an in house RNASeq analysis pipeline including adaptor trimming, QA, filtering and alignment of genome and transcriptome using TopHat2; and read-count based expression analysis was performed using DESeq2, and HTSeq and compared in each HSPC population between 4 DS and 3 normal FL samples. LOESS (Local polynomial regression fitting) was used for up/down regulated chromosomal domains

Results: Using ANOVA, 2713 genes were differentially expressed (DE) between HSPC populations from all 7 samples (FDR<0.001). Principal Component Analysis (PCA) using the top 300 DE genes showed good spatial segregation of HSPC into distinct lineage-specific populations in both normal and DS FL. The best spatial segregation was found using the top 300 DE genes which also showed that while PC1 separated normal and T21 HSPC equally, PC2 accounted for a

greater segregation of T21 than normal FL HSPC. Although lineage-specific gene expression patterns were largely maintained in DS HSPC, there were differences in levels of gene expression between DS and normal HSPC with the number of significantly (FDR<0.05) DE genes ranging from 10 in CMP to 1225 in CBP. Perturbation of lineage-associated gene expression was particularly prominent in CBP, HSC, MPP and MEP. More than half of all protein coding Hsa21 genes (154/243) were expressed in both DS and normal FL HSPC. Within each HSPC population, a consistent Hsa21 gene expression profile was seen in all 7 samples and most of the differences in Hsa21 gene expression between DS and normal HSPC were an exaggeration of the 'normal' HSPC profile rather than an aberrant expression of a different set of Hsa21 genes or a single Hsa21 gene. Using Loess smoothing, Hsa21 gene expression was consistently higher in DS HSPC compared to normal HSPC along the entire length of Hsa21 rather than in clearly defined domains. Finally, the majority of DE genes in all HSPC populations were located on chromosomes other than Hsa21.

Summary/Conclusion: These data show that global perturbation of fetal haematopoiesis by T21 is matched by genome-wide dysregulation of gene expression affecting most chromosomes in all HSPC, particularly lymphoid progenitors. Although DS FL HSPC populations all showed a 'T21 gene expression signature', this reflected expression of the same Hsa21 genes as in normal HSPC rather than being driven by aberrant expression of a small subset, or single, Hsa21 gene(s).

References: 1. Roy A et al, PNAS 2012 Oct 23;109(43):17579-84

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