Impact of trisomy 21 on the dynamics of human foetal haematopoiesis and leukaemia predisposition

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Principal supervisor's CRUK CC theme: Haematological Malignancies Programme

Department for student registration: Haematology
Department or institute where research will take place: Cambridge Stem Cell Institute, Jeffrey Cheah Biomedical Centre

Postgraduate scheme: MRes + PhD (1 + 3-year non-clinical applicants only)

MRes project outline:

It is now recognised that most paediatric blood cancers (leukaemias) have their origins during foetal life (Cazzola et al., Front. Cell Dev. Biol., 2021). MLL-fusion events, ETV6-RUNX1 and AML1-ETO translocations - responsible for acute lymphoid or myeloid leukaemias - have been detected in utero. In addition, 10% of neonates with trisomy 21 present with a transient acute megakaryoblastic leukemia, which can further progress into persistent acute myeloid leukaemia. Identifying the cellular origin of these cancers is critical to understand the biology of such diseases and eventually devise novel therapies. However, embryonic and foetal haematopoiesis is a complex and dynamic process, taking place in multiple waves and spanning several anatomical sites. It is still poorly characterised, especially in humans.

Our group has recently profiled human foetal haematopoietic tissues using single-cell transcriptomics, uncovering extended cellular heterogeneity across anatomical locations and over gestation time (Popescu et al., Nature, 2019; Jardine et al., Nature, 2021). We established a clonal tracking method that combines lentiviral barcoding (LARRY, Weinreb et al., Science, 2020) with in vitro culture of primary human haematopoietic stem and progenitor cells (HSPCs) isolated from foetal liver and neonatal cord blood tissues. We use single-cell RNA sequencing analysis to track how single HSPCs differentiate into all blood cell types and establish how specific differentiation behaviours are determined by specific HSPCs molecular states. Interestingly, we identified a novel haematopoietic stem cell (HSC) subset, uniquely present in foetal liver, with extensive proliferative capacity towards the erythroid and megakaryocyte lineages.

This rotation project will ask whether this foetal HSC subset also exist in foetal bone marrow and whether it is expanded in trisomy 21, potentially contributing to their leukemic predisposition.

MRes experimental plan:

This rotation project will comprise a mix of wet lab techniques and bioinformatic analysis methods. First, the student will be able to analyse LARRY scRNA-seq datasets previously generated in the laboratory (foetal bone marrow from disomic foetuses and foetal livers from both disomic and trisomy 21 foetuses). They will mine these scRNA-seq datasets, annotated with clonal relationships to identify HSPCs subsets based on their differentiation behaviours. This will include using bespoke Python or R code. They will also culture foetal liver HSPCs from disomic or trisomic foetuses to further validate the cellular behaviours of specific populations, checking

proliferation capacity, apoptosis rates, metabolic status, differentiation towards other blood lineages and other cellular parameters.

PhD project outline:

It is now recognised that most paediatric blood cancers (leukaemias) have their origins during foetal life (Cazzola et al., Front. Cell Dev. Biol., 2021). MLL-fusion events, ETV6-RUNX1 and AML1-ETO translocations - responsible for acute lymphoid or myeloid leukaemias - have been detected in utero. In addition, 10% of neonates with trisomy 21 present with a transient acute megakaryoblastic leukemia, which can further progress into a persistent acute myeloid leukaemia. Identifying the cellular origin of these cancers is critical to understand the biology of such diseases and eventually devise novel therapies. However, embryonic and foetal haematopoiesis is a complex and dynamic process, taking place in multiple waves and spanning several anatomical sites. It is still poorly characterised, especially in humans.

This PhD project aims to comprehensively characterise the dynamics of human foetal haematopoiesis at single cell resolution across anatomical sites and across gestation times. Given their predisposition to leukaemia, we will also characterise trisomy 21 foetuses, which present strongly enhanced erythropoiesis and megakaryopoiesis in utero (Roy et al., PNAS, 2012). The project will initially use an established clonal tracking method that combines lentiviral barcoding (LARRY, Weinreb et al., Science, 2020) with in vitro culture of primary human haematopoietic stem and progenitor cells (HSPCs) and sc-RNAseq. It will: 1) derive hierarchies of foetal HSPC differentiation and study relationships between HSPCs and their progeny; 2) study the kinetics of lineage expansion over time at the clonal level; 3) identify transcriptional signatures of specific expansion behaviours, in particular the enhanced propensity for Ery/Meg differentiation observed in Trisomy 21. Collectively, the project will provide novel insights into foetal haematopoiesis, which are essential for understanding how haematopoietic malignancies arise in utero in Trisomy 21 or in other contexts.

PhD experimental plan:

The PhD project will make use of both wet lab techniques and bioinformatic analysis methods. In a first phase the student will contribute to complete a set of LARRY barcoding experiments where human HSPCs from foetal or neonatal origin are differentiated along all main blood lineages. Tissues examined will include foetal liver, foetal bone marrow or cord blood from disomic foetuses or neonates respectively, and foetal liver and foetal bone marrow from trisomy 21 foetuses. Lineages read-outs will be scRNAseq and multiparameter flow cytometry.

The students will then analyse these functional and scRNA-seq datasets with bioinformatic methods to identify: i) clonal relationships between lineages; 2) which transcriptional signatures of HSPCs correspond to specific differentiation behaviours; 3) identify cellular and molecular differences that underlie the different lineage output observed in all the tissues studied; 4) identify the cellular and molecular basis of the increased Ery/Meg output observed in Trisomy 21.

They will then validate their findings prospectively purifying specific populations and checking proliferation capacity, apoptosis rates, metabolic status, differentiation towards other blood lineages and other cellular parameters. Xenograft transplantation of specific subsets in immunocompromised mice will also be used to assess their self-renewal potential and lineage potential in vivo.

Main techniques:

- Multiparameter flow cytometry
- Isolation and culture of primary human haematopoietic stem and progenitor cells
- Lentiviral vector production and transduction
- Single cell transcriptomics
- Lineage tracing with the LARRY method
- Bioinformatic analysis, Python and R coding
- Xenograft transplantation of human haematopoietic stem and progenitor cells into immunocompromised mice

Key references:

- Cazzola et al., Front. Cell Dev. Biol., 2021: https://www.frontiersin.org/articles/10.3389/fcell.2020.618164/full
- Popescu et al., Nature, 2019: https://www.nature.com/articles/s41586-019-1652-y
- Jardine et al., Nature, 2021: https://www.nature.com/articles/s41586-021-03929-x
- Weinreb et al., Science, 2020: https://science.sciencemag.org/content/367/6479/eaaw3381
- Roy et al., PNAS, 2012 : https://www.pnas.org/doi/10.1073/pnas.1211405109