LATE-BREAKING ORAL PRESENTATIONS

2034 – RAB5C-MEDIATED ENDOCYTIC TRAFFICKING REGULATES HEMATOPOIETIC STEM AND PROGENITOR CELL DEVELOPMENT THROUGH NOTCH AND AKT SIGNALING

Feng Liu
Institute of Zoology, Chinese Academy of Sciences, Beijing, China (People's Republic)

A better understanding of molecular mechanisms underlying hematopoietic stem and progenitor cell (HSPC) development may lead to improved protocols to generate functional and transplantable HSPCs ex vivo. Here we report that Rab5c is essential for HSPC specification by endocytic trafficking of Notch and AKT signaling in zebrafish embryos. Rab5c deficiency leads to defects in HSPC production. Mechanistically, Rab5c regulates hemogenic endothelium specification by endocytic trafficking of Notch ligand and receptor. We further show that the interaction between Rab5c and Appl1 in endosome is required for the survival of endothelial cells in the aorta-gonad-mesonephros region through AKT signaling. Interestingly, Rab5c over-activation can also lead to defects in HSPC production, which is attributed to excessive endolysosomal trafficking inducing Notch signaling defect. Taken together, our findings establish a previously unrecognized role of Rab5c-mediated endocytic trafficking in HSPC development, and provide new insights into how spatiotemporal signals are orchestrated to accurately execute cell fate transition.

2035 – AGE-RELATED DOWNREGULATION OF LMNA IMPACTS HUMAN HEMATOPOIETIC STEM CELL FUNCTION

Hsuan-Ting Huang; Emmalee Adelman; Dean Wade; Gabriel Gaidosh; Ramin Shiekhattar; Maria Figueroa
University of Miami, Miami, United States

Hematopoietic stem cells, essential for the production of blood cells, decline in function with aging. Among these changes with aging are increased frequency of mutations in epigenetic modifiers. We analyzed the epigenetic landscape of Lineage-CD34+CD38- human HSC-enriched fraction (HSCe) from young (18-30 years), middle-aged (45-55 years), and aged (65-75 years) individuals by comparing changes in gene expression by RNA sequencing and histone marks (H3K4me3, H3K4me1, H3K27ac, H3K27me3) by ChIP sequencing. Integrative analysis of the histone profiles by k-means clustering identified 12 clusters of genes defined by their specific pattern of age-associated epigenetic changes, which were accompanied by changes in gene expression affecting 1,133 genes between young and aged HSCe. The LMNA gene, which encodes the nuclear lamina protein Lamin A/C, was one of the most downregulated genes with aging (7.9-fold, \( p = 1.9 \times 10^{-13} \)). This downregulation was mediated by loss of active histone marks H3K4me3 at the LMNA gene promoter, as well as loss of H3K27ac at two putative enhancer regions. ShRNA-mediated knockdown of LMNA in young, CD34+ hematopoietic stem and progenitor cells (HSPCs) resulted in an increase in myeloid colony formation (\( n = 7 \) biological replicates with an average of 33% in total colony increase), consistent with the aging phenotype. Moreover, knockdown of LMNA in CD34+ cells impaired myeloid differentiation in liquid culture as determined by persistence of CD34 expression (\( n = 5, \ p \leq 0.0001 \)) with a delay in CD11b expression (\( n = 5, \ p \leq 0.05 \)). Initial results by STORM super resolution imaging show loss of LMNA leads to enlarged nuclear size and changes in chromatin localization. In conclusion, loss of LMNA recapitulates features of HSC aging, and restoring LMNA levels may be important for restoring normal function in aged HSCs.
T-cell acute lymphoblastic leukemia (T-ALL) can affect any age group; however, disease outcome is worse in older patients. Aging has been described to be accompanied by an increase in the body's proinflammatory status, a phenomenon referred as “inflammaging”. By analysing two T-ALL transcriptome datasets, we have found that inflammation pathways are upregulated in older patients, whereas younger patients present upregulation of cell cycle pathways, suggesting that inflammaging is also recapitulated in T-ALL. Here, we hypothesize that T-ALL oncogenes can cooperate with specific cytokines/stimuli and their signaling pathways to create distinct leukemic phenotypes. To test this, human cord blood CD34+ cells were transduced with different T-ALL oncogenes and grown in co-culture with stromal cells under different pro-inflammatory conditions. We observed that most of the transduced cells had their cell growth negatively affected by the proinflammatory conditions, although some oncogene combinations showed more tolerance to these conditions than others. Activated NOTCH1+TLX3 transduced cells represented the only gene combination that showed significantly higher cell growth in the presence of interferon-gamma (IFNγ) when compared to non-transduced cells in the same culture. IL-15 and IFNγ potentiated differentiation arrest observed with certain oncogene combinations. Normal uncommitted T-cell progenitors (CD7+CD5+CD44+CD1a-) responded to IL-15 via STAT5 and AKT phosphorylation, and this response was enhanced by chronic treatment with IL-15. Our results suggest that proinflammatory conditions can alter the effect of certain oncogenes on T-cell progenitors and influence leukemogenesis, potentially leading to distinct biological behaviors of resulting leukemias. These findings may lead to identification of cytokine dependencies associated with particular T-ALL genetic subtypes.
**3166 – THE ROLE OF AUTOPHAGY IN HEMATOPOIESIS AND HEMATOPOIETIC DISEASE**

*Alvin Ma¹, XK Chen²*

¹The Hong Kong Polytechnic University, Hong Kong, Hong Kong; ²The University of Hong Kong, Hong Kong, Hong Kong

Autophagy is a highly regulated cellular process essential for the maintenance of cytoplasm and clearance of expired proteins. While it is widely believed that autophagy is a two-edged sword during oncogenesis, controversial results were also reported in leukemia, suggesting that autophagy also plays a complex role in leukemogenesis, depending on cell context and stages of leukemogenesis. Nevertheless, a single in vivo model to prove the hypothesis is lacking and the role of autophagy in normal hematopoiesis remains largely unknown. Here we make use of the zebrafish model to investigate the role of autophagy in hematopoiesis under normal and pathological conditions.

We targeted ulk1b in both wild-type and FLT3-ITD overexpressing (FLT3ITD) zebrafish embryos. Zebrafish ulk1b somatic mutant embryos in both WT or FLT3ITD background displayed defective autophagy as shown by Lc3-I/II level as well as in vivo LysoTracker and LysoSensor staining. Defective autophagy associates with increased myelopoiesis in wild-type embryos and more importantly, the further enhanced pre-leukemic myeloproliferation in zebrafish FLT3ITD model. Interestingly, targeting ulk1b ameliorated the persistent myeloproliferation observed in FLT3ITD model, demonstrated the complex role of autophagy during leukemogenesis. In both wild-type and FLT3ITD embryos, targeting ulk1b also decreased p53 and increased
mTOR, providing a mechanistic explanation for myeloproliferation and suggested a previously undescribed bi-directional regulatory role between autophagy and mTOR/p53.

In summary, we make use of the strength of zebrafish model to demonstrate the complex role of autophagy in normal and malignant myelopoiesis. Further studies are warrant to examine the undefined role of autophagy in leukemogenesis, which is important for the development of effective autophagy-related therapeutic strategies against AML.

3167 – DEVELOPMENTAL CHANGES IN THE CHROMATIN ACCESSIBILITY OF HEMATOPOIETIC STEM CELLS ARE ASSOCIATED WITH DISTINCT GENETIC PROGRAMS

Trine Ahn Kristiansen1; Jonas Ungerbäck1; Niklas Krausse1; Petter Säwen1; Stefano Vergani1; David Bryder2; Mikael Sigvardsson3; Joan Yuan1

1Department of Molecular Hematology, Lund Stem Cell Center, Medical Faculty, Lund University, Lund, Sweden, Lund, Sweden; 2Department of Molecular Hematology, Lund Stem Cell Center, Medical Faculty, Lund University, Lund, Sweden, Department of Microbiology and Immunology, Institute of Biomedicine, Gothenburg University, Gothenburg, Sweden, Lund, Sweden; 3Department of Molecular Hematology, Lund Stem Cell Center, Medical Faculty, Lund University, Lund, Sweden, Institution for Clinical and Experimental Sciences, Faculty of Health Sciences, Linköping University, Linköping, Sweden, Lund, Sweden

Hematopoietic stem cells (HSCs) undergo a developmental switch in neonatal mice hallmarked by a decrease in self-renewing divisions and entry into quiescence. Molecular determinants like dynamic changes in the chromatin landscape across the switch hold key insights into developmental changes in cell fate and function but remain poorly understood. Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq) has emerged as a powerful tool to study chromatin accessibility in rare cells and in combination with genome-wide expression profiling has the potential to resolve cell specific transcriptional networks.

To explore the regulation of the switch between fetal liver and adult bone marrow HSC states as well as during the first stages of hematopoietic differentiation, we here assess the changes in chromatin accessibility and genome-wide gene expression patterns in immunophenotypically defined HSCs and lymphoid primed multipotent progenitors (LMPPs). Chromatin accessibility unique to adult bone marrow HSCs were found to be enriched for putative binding sites of the megakaryocyte and erythroid lineage transcription factors Nuclear factor erythroid 2 (NFE2). In parallel, adult HSC gene expression and epigenetic profiles were highly enriched for the megakaryocyte gene signature which is strikingly absent in the E14.5 fetal liver. Taken together, our results identify a previously unrecognized hallmark of the developmental switch of the HSC compartment centered around acquisition of a megakaryocyte bias. These age specific features may have important implications in host response to environmental insults.

3168 – INVESTIGATING THE ROLE OF THE KDM4A LYSINE DEMETHYLASE IN PEDIATRIC ACUTE MYELOID LEUKEMIA

Frederick A. Mallette1; Christina Sawchyn1; Florence Couteau2; Marie-Eve Lalonde3; Karine Boulay4; Dagmar Glatz5; Erlinda Fernandez-Diaz1; Johannes Zuber6; Sonia Cello1; Francois Mercier7

1Université de Montréal, Montreal, Canada; 2Université de Montréal / CR-HMR, Montreal, Canada; 3Université de Montreal, Montreal, Canada; 4Université de Montréal, Montreal, Canada; 5Maisonneuve-Rosemont Hospital Research Center, Montreal, Canada; 6Research Institute of Molecular Pathology, Vienna, Austria; 7McGill University, Montral, Canada
In Canada, almost one third of childhood cancers are leukemias. Encouragingly, many of these pediatric leukemias are now treatable with a variety of anti-cancer drugs. Nonetheless, leukemia still accounts for an alarming ~25% of cancer-related deaths in children. The goal of therapy against leukemia is to kill malignant cells while sparing normal hematopoietic stem and progenitor cells (HSPC). Recent research has revealed multiple events occurring at the genome level that promote transformation of HSPC. These events include genetic alterations (DNA mutations, chromosomal translocations), as well as epigenetic modifications, leading to alteration of the gene expression pattern of HSPC. Interestingly, genes coding for epigenetic regulators are frequently mutated, deleted or amplified in leukemia. We have identified that a specific chromatin modifier, the lysine demethylase KDM4A, is overexpressed in pediatric MLL-AF9 acute myeloid leukemia, and plays essential roles for the proliferation and survival of leukemic cells, but dispensable for normal HSPC. The main objective of the proposed research project is to decipher the precise molecular mechanisms by which this epigenetic regulator promotes progression of pediatric AML using innovative cellular, molecular, genetic, and pharmacological approaches. The proposed studies have great potential to not only allow a better understanding of molecular events underlying pediatric AML, but also identify effective drugs for combating childhood blood cancer that is still plagued with dismal survival rates.

3169 – ONCOGENE CONTRIBUTIONS IN A SYNTHETIC MODEL OF DE NOVO GENERATED HUMAN T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA

Ann Chong Sun¹; Manabu Kusakabe²; Kateryna Tyshchenko²; Kelly Wei²; Emmanouil Kyroglou²; Tracy Huynh²; Gilmar Gutierrez²; Aastha Nanda²; Sam Gusscott²; Keith Humphries²; Martin Hirst³; Connie Eaves²; Andrew Weng²

¹University of British Columbia, Burnaby, Canada; ²Terry Fox Laboratory, British Columbia Cancer Agency, Vancouver, Canada; ³University of British Columbia, Vancouver, Canada

T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive malignancy with diverse oncogenic drivers, the relative contributions of which remain obscure. Enforced expression of select oncogenes is sufficient to generate T-ALL in mice, however, mouse models are limited in their value for understanding human disease. We recently developed the first “synthetic” model of human T-ALL by lentiviral transduction of normal human CD34+ cord blood progenitors with a combination of four known T-ALL oncogenes: NOTCH1, LMO2, TAL1, and BMI1 (NLTB). These cells expand robustly in culture and produce aggressive, serially transplantable T-ALL in immunodeficient mice. Additionally, we found that LTB alone fails to perform in vitro or in vivo, highlighting an essential role of NOTCH1. To determine the minimal complement of oncogenes required to generate de novo human T-ALL, we executed a “leave-one-out” and “leave-two-out” strategy. We scored the following oncogene combinations in vitro and in vivo: NLB, NLT, NTB, NL, NT, and NB. We found that the various oncogene combinations yielded a spectrum of aberrant phenotypes affecting cell differentiation and proliferation, and a subset produced aggressive leukemias in mice. We also performed RNA-seq on non-leukemogenic and preleukemic cell populations, and fully transformed leukemic cells to define gene expression programs necessary for cellular transformation. Our approach allows us to attribute specific gene programs and cellular phenotypes to each oncogene individually and in combination. Our synthetic approach is flexible, reproducible, and experimentally tractable, allowing functional testing of individual genetic variants and can also serve as a customizable platform for testing of targeted therapeutics.

3170 – ABERRANT BONE MARROW MICROENVIRONMENT IN THERAPY RELATED MYELOID NEOPLASM

Monika Kutyna¹; Sharon Paton¹; Stan Grontos¹; Devendra Hiwase²

¹University of Adelaide, Adelaide, Australia; ²Royal Adelaide Hospital/University of Adelaide, Adelaide, Australia
**Introduction:** Therapy related myeloid neoplasm (t-MN) is a lethal second hematological malignancy following chemotherapy (CT) and radiotherapy (RT) for primary cancers. It accounts for 15-20% of myelodysplastic syndromes (MDS) and acute myeloid leukemia. Prognosis of t-MN is poor with no effective therapies. Although CT/RT can damage the bone marrow microenvironment, very limited studies assessed it in t-MN.

**Aim:** To compare bone marrow microenvironment milieu and mesenchymal stromal cells (MSC) from primary MDS (pMDS), t-MN and double cancers (DC, primary cancer and MDS but without a history of CT/RT for primary cancer).

**Methods:** Bone Marrow plasma cytokines/chemokines (CCK) and bone marrow MSC proliferation, senescence, morphology and differentiation potential of pMDS, t-MN and DC was compared. **Results:** Proliferation and clonogenic potential of MSC from t-MN were significantly lower than pMDS and DC (Fig.1A). MSC from t-MN could not be maintained in culture and had higher senescence rates, assessed by β-galactosidase positive cells, compared to pMDS and DC (p<0.001) (Fig.1B). T-MN MSC were also larger associated with increased filamentous actin, compared to pMDS (p<0.0001) (Fig.1C). Ability to form a mineralized matrix was increased in t-MN (p<0.03) MSC, while adipogenic potential was significantly reduced compared to pMDS and DC (p<0.03) (Fig.1D). CCK profiling of bone marrow from t-MN and pMDS showed differential expression of 18/25 CCK (Fig.2).

**Conclusions:** Our data demonstrate that MSC from t-MN patients have significantly altered morphology, a lower proliferation rate, and higher senescence rate compared to pMDS and DC. Osteogenic differentiation of t-MN MSC was higher at the cost of reduced adipogenic differentiation. This data suggests that RT/CT leads to long term damages to bone marrow microenvironment which may play a role in t-MN pathogenesis.

**3173 – AP2A2 KO MICE LINK FETAL LIVER HAEMATOPOIESIS EXHAUSTION TO LOSS OF HSC QUIESENCE, PERTURBED ASYMETRICAL FATE AND ALTERED LIPID METABOLISM.**

*Stephen Ting*¹; *Sara Rhos*²; *Jesslyn Saw*²; *David Curtis*²; *Graham Magor*²; *Andrew Perkins*²

¹Eastern Health, Haematology Department, Melbourne, Australia; ²Australian Centre for Blood Diseases, Melbourne, Australia

We identified Ap2a2 as an enhancer of mouse long-term (LT-) HSC function via a bone marrow (BM) transplantation screen, and a HSC cell fate determinant during live cell HSC videomicroscopy. We hypothesised Ap2a2-transduced HSCs acquired in-vitro quiesence. Using the Tet-On H2B-GFP mouse line - We transduced vector versus AP2A2 into H2B-GFP HSCs for an in-vivo pulse-chase transplantation assay. This showed a 3-fold increase in the dormant GFP-high (CD150+48-LSK) LT-HSC subpopulation of Ap2a2-transduced-HSC recipients. We present two new mouse transgenic lines: an Ap2a2-LacZ reporter where X-Gal/FDG staining of BM cells confirmed higher endogenous Ap2a2 expression in LT- versus short-term (ST- CD150-48-LSKs) HSCs and a constitutive Ap2a2 knock-out (KO) line. Analyses of heterozygote Ap2a2 KO matings showed less than expected 25% Ap2a2-/- Mendelian inheritance at weaning consistent with fetal and peri-natal lethality. E14. 5 Ap2a2-/- embryos had smaller livers but twice as many LT-HSCs, confirmed by limit dilution assay. Ki67/DAPI cell cycle analyses showed loss of G0 in E14.5 LT- versus ST-HSCs. At E16.5-E18.5, there were two embryonic phenotypes: Ap2a2-/- survivors defined by restoration of FL LT-HSC G0 and differentiation as opposed to Ap2a2-/- non-survivors with continued loss of G0 and failed differentiation. Numb staining of Ap2a2-/- E14.5 LT-HSCs showed perturbed asymmetrical to
symmetrical divisions. RNAseq analyses of Ap2a2-/- compared to Ap2a2+/+ E14.5 LT-HSCs showed
differential upregulation of PPAR and lipid signaling pathways. Functionally, E14.5 Ap2a2-/- FL cells have
maintained splenic colony formation but significantly impaired competitive transplant reconstitution. We
conclude constitutive Ap2a2 deletion results in FL HSC exhaustion via loss of LT-HSC G0 quiescence and
differentiation, which mechanistically involves the PPAR-lipid metabolism pathways.

3174 – TANDEM MYELOID GROWTH FACTOR TREATMENT ENHANCED THE TOTAL
HAEMATOPOIETIC STEM CELL (HSC) POOL AND MAGNITUDE OF HSC MOBILISATION.

Anuj Sehgal1; Lena Batoon1; Simranpreet Kaur1; Susan Millard1; Allison Pettit1; Liza Raggatt2; Andy Wu1
1Mater Research Institute - UQ, Brisbane, Australia; 2Mater Research Institute, Brisbane, Australia

Autologous HSC transplant is a common therapeutic strategy for some haematological malignancies but is
unachievable in up to 40% of cases due to insufficient mobilisation of HSCs for successful transplantation.
The monocyte-macrophage colony stimulating factor-1 (CSF1) improves HSC transplantation outcomes in
preclinical models and clinical trials, but the underlying mechanism of CSF1 induced benefit is unclear. BM
macrophages are a key component of the HSC niches and a primary target of granulocyte colony-stimulating
factor (G-CSF) induced HSC mobilisation. We examined CSF1 impacts on HSC frequency using a modified
CSF1-Fc molecule that has improved drug qualities. Haematopoietic progenitor cells (HPC), but not HSCs or
multipotent progenitors (MPP), expressed the CSF1 receptor. Acute CSF1-Fc treatment (4 x daily injection)
induced a robust myeloproliferative response that was evident 1 week post the initial injection. At this time,
BM HSCs (17 fold) and MPP (19 fold), but not HPC, were significantly reduced. However, at 2 weeks post
the first CSF1-Fc injection, after the myeloproliferative event had resolved, the overall pool of HSCs in BM
(1.6 fold), and unexpectedly spleen (9.2 fold), was significantly elevated compared to controls. A mobilising
regimen of G-CSF started at 2 weeks post CSF1-Fc treatment enhanced HSC mobilisation (2.5 fold) into
blood when compared to mice treated with G-CSF only. This increase in mobilised HSCs was verified via
competitive secondary transplantation. The data suggest that CSF1 has complex indirect effects on HSCs to
increase HSC frequency and promote formation of extramedullary HSC niches. CSF1-induced increased in
the total HSC pool may contribute to the reported improvement in transplantation outcomes associated with
CSF1 therapy and reveals a novel strategy to increase HSCs prior to mobilisation.

3175 – HEMATOPOIETIC STEM CELL-DEPENDENT NOTCH TRANSCRIPTION IS MEDIATED
BY P53 THROUGH THE HISTONE CHAPERONE SUPT16

Yoonsung Lee
Institute for Basic Science, Ulsan, Republic of Korea

Hematopoietic stem cells (HSCs) are rare cells that can self-renew and differentiate into all blood cell
lineages for life. HSCs have long been the focus of developmental and regenerative studies, yet our
understanding of the signaling events regulating their specification remains incomplete. In order to identify
novel genes and transcription factors involved in hematopoietic specification, we performed a forward
genetic screen to identify zebrafish mutants defective in HSC formation. Through large-scale whole mount in
situ hybridization based screens followed by RNA-sequencing-based linkage mapping, we
identified that supt16h, a component of the FAcilitates Chromatin Transcription (FACT) complex, is
required for HSC formation. Zebrafish supt16h mutants express reduced levels of Notch signaling
components, genes essential for HSC development, due to abrogated transcription. Although cellular
functions of Supt16h is generally known for regulating transcription and reorganizing nucleosomes to alter
chromatin accessibility, global chromatin accessibility in the zebrafish supt16h mutants is unaffected.
However, we observe a specific increase in accessibility at the p53 locus leading to an accumulation of P53
protein in the supt16h mutants and abrogation of increased p53 levels in supt16h mutants rescues both loss of
Notch and HSC phenotypes. We further demonstrate that P53 levels directly influence expression of the Polycomb Group protein, Phc1, which functions as a transcriptional repressor of Notch genes. Suppression of phc1 or its upstream regulator, p53, rescues both loss of Notch and loss of HSC phenotypes in supt16h mutants. Taken together, our results highlight a previously uncharacterized relationship between supt16h, p53, and phc1 to specify HSCs via modulation of Notch signaling.

3176 – MULTIPOTENT RAG1+ PROGENITORS EMERGE DIRECTLY FROM HEMOGENIC ENDOTHELIUM IN HUMAN PSC DERIVED HEMOPOIETIC ORGANOIDS

Ali Motazedian1; Freya Bruveris1; Santhosh Kumar1; Jacqueline Schiesser1; Tyrone Chen2; Elizabeth Ng1; Ann Chidgey3; Christine Wells2; Andrew Elefanty1; Edouard Stanley1

1Murdoch Children's Research Institute, Melbourne, Australia; 2The University of Melbourne, Melbourne, Australia; 3Monash University, Melbourne, Australia

Defining the ontogeny of the human adaptive immune system during early embryogenesis has implications for understanding childhood diseases including leukemias and autoimmune diseases. Using RAG1:GFP human pluripotent stem cell reporter lines, we examined human T-cell genesis from pluripotent stem cell derived hematopoietic organoids. Under conditions favouring T-cell development, RAG1+ cells progressively up regulated a cohort of recognised T-cell associated genes, arresting development at the CD4+CD8+ stage. However, sort and re-culture experiments showed that early RAG1+ cells also possessed B-cell, myeloid and erythroid potential. Moreover, RNAseq analysis of the early RAG1+ cells indicated that, in addition to T-lineage genes, this population expressed genes associated with erythroid and myeloid lineages, as well as genes marking endothelium and hematopoietic progenitors. Consistent with this, imaging studies, flow cytometry and single cell RNAseq data showed that the first RAG1+ cells emerged directly from SOX17+ endothelial structures and co-expressed CD90, CDH5 and the endothelial marker, CAV1. These observations provide evidence for a wave of human T-cell development that originates directly from hemogenic endothelium via a RAG1+ intermediate with multilineage potential.

3177 – THE LARGE-SCALE GENERATION OF MATURE, HEMOGLOBINIZED RED BLOOD CELLS IN VITRO FROM HUMAN PLURIPOTENT STEM CELLS FOR DISEASE MODELING AND AUTOLOGOUS THERAPIES

Ashlee Conway1,2; Tolulope Roswano2; Thomas Williamson2; Martha Clarke3; Melissa Kinney2; Trista North4; George Daley4

1Harvard Medical School, Boston, United States; 2Boston Children's Hospital, Boston, United States; 3Harvard TH Chan School of Public Health, Boston, United States; 4Harvard Medical School Stem Cell Institute, Boston, United States

Human induced pluripotent stem cells (iPSC) are an invaluable resource in tissue and blood cell engineering due to their multi-lineage potential in culture systems. iPSC-derived progenitors that undergo induced erythropoiesis in vitro would allow for the modeling of blood diseases, such as Sickle Cell Anemia (SCA), for therapeutic applications, as well as the possibility of becoming an autologous source of blood products for patients. The robust generation of terminal, mature red blood cells (RBCs) in large numbers from iPSCs has historically been challenging, due to a lack of terminal erythropoietic development. Here, we describe an optimized method of generating terminal RBCs in vitro from healthy and Sickle homozygous patient-derived iPSCs using a plasma-rich culture media and hypoxic conditioning. Committed erythroid cells underwent prominent proliferation from an enriched CD34+ population (90-fold amplification), as well as efficient adult globin switching (>40%), and enhanced rates of enucleation (>60%). In their terminal state, RBCs with the Sickle homozygous mutation displayed morphological and pathological characteristics that matched the
3180 – INVESTIGATING IRRADIATION DAMAGE TO HAEMATOPOIETIC NICHES IN THE SPLEEN

Christie Short1; Helen O’Neill2; Jonathan Tan1
1Bond University, Gold Coast, Australia; 2Bond Unievrsity, Gold Coast, Australia

Haematopoietic stem cell transplantation (HSCT) is used to treat patients with blood disorders such as leukaemia. Intravenous HSCT allows cells to home to the bone marrow niche and initiate long-term haematopoietic reconstitution. However, HSC can also home to extramedullary organs such as the spleen. Under these circumstances, the spleen possesses the capacity to support extramedullary haematopoiesis (EMH) and immune cell production. This secondary function of the spleen could be targeted to enhance early haematopoietic reconstitution after HSCT. To ensure the success of HSCT, patients must first be conditioned to clear niche space for transplanted HSC to engraft. Radiotherapy used to condition HSCT recipients not only causes myeloablation but also results in non-specific damage to multiple tissues including bone marrow and stem cell niches. It is known that sinusoidal endothelial cells in the bone marrow are both important HSC niche regulators and are damaged following irradiation. In the murine spleen, Scf-expressing endothelial cells and Tcf21+ cells have been shown to form perisinusoidal niches in the red pulp for HSCs to reside in. This project investigates whether damage caused by lethal irradiation extends to the spleen, by examining stromal and endothelial cells using markers such as CD105, MAdCAM-1, MECA-32, VCAM-1, PDGFRβ and CD31 in order to resolve multiple vascular and mesenchymal structures including splenic HSC niches. If the stromal niches regulating extramedullary haematopoiesis during the early period of haematopoietic recovery can be better understood, it may lead to therapies which accelerate immune reconstitution and ultimately lessen rates of transplant-associated mortality.

3182 – PHARMACOLOGICAL INHIBITION OF NEMO-LIKE KINASE RESCUES MTOR-MEDIATED TRANSLATION AND ERYTHROPOIESIS IN PRE-CLINICAL MODELS OF DIAMOND BLACKFAN ANEMIA

Mark Wilkes1; Jaqueline Mercado2; Mallika Saxena2; Jun Chen3; Kavitha Siva3; Gianlucca Varetti4; HeeDon Chae2; Minyoung Youn2; Hanna Gazda5; Manuel Serrano4; Johan Flygare3; Kathleen Sakamoto2
1Division of Hematology/Oncology, Pediatrics Department, Stanford University, Stanford, United States; 2Stanford University, Stanford, United States; 3Lund University, Lund, Sweden; 4Institute for Research in Biomedicine, Barcelona, Spain; 5Harvard Medical School, Boston, United States

Diamond Blackfan Anemia (DBA) is associated with anemia, congenital abnormalities, and cancer. Current therapies for DBA have undesirable side effects, including iron overload from repeated transfusions or infections from immunosuppressive drugs and stem cell transplantation. Nemo-like Kinase (NLK) is hyperactivated in erythroid progenitors in murine and human models as well as DBA patients. In an RPS19-insufficient human model, genetic silencing of NLK increased erythroid expansion by 2.2 fold, indicating that aberrant NLK activation contributes to disease pathogenesis. A high-throughput inhibitor screen identified a compound that inhibits NLK (IC50:440nM) and increased erythroid expansion in murine (5.4 fold) and human (6.3 fold) models of DBA with no effect on wild type erythropoiesis (EC50: 0.7 µM). Virtually identical results were observed in CD34+ progenitors from 3 DBA patient bone marrow aspirates with 2.3, 1.9 and 2.1 fold increases in CD235+ erythroblast generation.
In erythroid progenitors, RPS19 insufficiency increased phosphorylation of the mTORC1 component Raptor, reducing mTOR activity by 82%. This was restored to basal levels upon inhibition of NLK. To compensate for a reduction in ribosomes, stimulating mTOR activity with leucine has been proposed to increase translational efficiency in DBA patients. Probably due to NLK phosphorylation of raptor, DBA patients did not respond as anticipated. While leucine treatment mildly increased mTOR activity in both control and RPS19-insufficiency, combining leucine with NLK inhibition increased mTOR activity to 142% of control and significantly improved erythroid expansion.

Identification of aberrantly activated enzymes, such as NLK, offer therapeutic promise used alone, or in combination with existing therapies, as druggable targets in the clinical management of DBA.

3183 – THE ROLE OF THE THROMBOPOIETIN RECEPTOR (MPL) IN JAK2V617F-POSITIVE MYELOPROLIFERATIVE NEOPLASMS

Kira Behrens¹, Elizabeth Viney¹; Tracy Willson¹; Jeff Babon¹; Nicos Nicola¹; Warren Alexander¹

¹The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia; ²Department of Medical Biology, University of Melbourne, Parkville, Australia

Constitutive activation of the Jak/Stat signalling pathway is a hallmark of Myeloproliferative Neoplasms (MPN), with 60-90% of patients carrying the activating JAK2 mutation, JAK2V617F. Several studies suggest expression of a type I cytokine receptor is essential for JAK2V617F-mediated transformation, but the precise contribution of such receptors is unresolved. Here we investigate the functional role of Mpl in JAK2V617F-driven MPN in vitro and in vivo. In vitro studies confirmed that Mpl expression was required for JAK2V617F-induced transformation, but identified a Mpl truncation mutant retaining the JAK2-interaction domain, but lacking the C-terminus (Mpl-IC36), typically associated with Stat5 binding, to be sufficient to support JAK2V617F-induced transformation. Interestingly, Stat5-phosphorylation was maintained. In contrast, deletion of the entire cytoplasmic receptor domain abrogated Stat5 phosphorylation and factor-independent proliferation. In vivo Mpl-wt;JAK2V617F mice exhibited the thrombocytosis and erythrocytosis typical of MPN, while Mpl deficiency abrogated thrombocytosis but sustained high red blood cell counts. Although the Mpl-IC36 receptor was sufficient for JAK2V617F-induced factor independence in BaF/3 cells, Mpl-IC36;JAK2V617F mice did not develop thrombocytosis, indeed platelet counts were similar to those in Mpl null mice. This underlines differences that may emerge from in vitro and in vivo systems and highlights the important role of the Mpl cytoplasmic region for JAK2V617F-mediated signalling, extending the function of Mpl in JAK2V617F-induced MPN beyond a simple scaffold for active JAK2.