Invited Speakers

Session 13
Differentiation diversity and arrest in the myeloid lineage (1032)
David Scadden, Massachusetts General Hospital, CRM, USA

The changes that hematopoietic cells undergo as they move from primitive stem/progenitors to mature effector cells are increasingly well defined in molecular terms. Perturbation of this process is a hallmark of leukemias. We tested aberrant expression of regulatory molecules that must be silenced for myeloid differentiation to occur and demonstrated that they induced a differentiation blockade mimicking AML. Using this system, we defined genes and small molecules that can overcome differentiation blockade. One of these was further pursued and shown to target a metabolic pathway not previously shown to regulate differentiation, the uridine synthesis pathway. Inhibiting it pharmacologically resulted in marked improvement in animal survival and leukemic cell differentiation in mouse models of AML. These data suggest that molecularly driven models of differentiation arrest can be productively used to identify unanticipated biochemical events regulating differentiation and define possible therapeutics.

The method of arresting myeloid differentiation can also be used to expand primitive populations and characterize them once differentiation blockade is paused and maturation proceeds. Since the undifferentiated cells can be expanded on a clonal basis, we separated individual GMP-like progenitors and assessed the functional attributes of their daughter cells. Notably, GMP progenitors were either granulocyte or monocyte competent, but no clone was bipotent for granulocytes and monocytes. Further, monocyte function was highly heterogeneous and stable in a clone-specific manner. For example, monocytes had clone-specific ability to respond to or kill specific types of microbes. These data indicate unexpected functional variability among innate immune cells where descendents of an individual progenitor have a signature set of functions that is invariant. Innate immunity may be less the indiscriminant killers than common wisdom presumes and represents a collection of highly specific functional cell types.
**Abstract 3214**

**Aspirin Treated Essential Thrombocythemia Patients: Which Timing?**

The essential thrombocythemia (ET) is a myeloid neoplasm characterized by platelet hyperreactivity and thrombotic risk. The treatment with aspirin (ASA) is recommended in ET patients at risk of first-time or recurrent thrombotic events. An unexplored topic is the optimal timing of once daily ASA intake. On the basis of the presumptions that 1) platelet aggregation is higher in the morning and that 2) the platelet inhibitory effect of ASA is not sustained during the usual 24-hour (h) dosing interval and that 3) a higher gastric mucosal resistance in the evening, we evaluated platelet count, β-thromboglobulin (β-TG) and platelet factor 4 (PF4), as markers of platelet activation, the platelet function activity (PFA), as indicator of ASA platelet sensitivity. We studied 60 patients (20 men, 40 women; mean age 51 years, range 32-70) with ET according to WHO criteria. The mean duration of disease was 11 years. All patients were on ASA 100 mg once daily. Of these, 30 took ASA on awakening and 30 took ASA at bedtime. Of the 60 patients, 45 were on anagrelide hydrochloride (daily dose 1.5 mg) (10 men, 35 women), 15 were on hydroxyurea (daily dose 2 mg) (10 men 5 women). None had inherited or acquired thrombotic risk factors. Sixty subjects served as controls. Platelets were measured by automated analyzer. β-TG and PF4 were determined by ELISA. ASA platelet sensitivity was measured by Platelet Function Analyzer (PFA-100). The mean platelet count was 455±200 x10⁹/L. The awakening ASA patients had normal β-TG and PF4 (12±5 IU/ml and 4±1 IU/ml) and prolonged C/EPI closure time (T, unit: s, n.v. 84-160 s) (249±40 s), whereas the bedtime ASA patients had high β-TG and PF4 (200±15 IU/ml vs 20±11 IU/ml and 170±50 IU/ml vs 6±2 IU/ml, respectively) (p<.0001 and p<.0001, respectively) and normal C/EPI closure time (T, unit: s, n.v. 84-160 s) (90±10 s). These findings suggest that in ET patients the optimal timing of once daily ASA intake is in the morning.

**Late-breaking Abstracts – Short Talks**

*(confirmed after 31 July)*

**2019 – ENDOCARDIALLY-DERIVED MACROPHAGES ARE ESSENTIAL FOR THE REMODELING OF HEART VALVES**

*Austin Nakano¹; Ayako Shigeta¹; Vincent Huang¹; Jonathan Zuo¹; Rana Besada¹; Yasuhiro Nakashima¹; Yan Lu¹; Matteo Pellegrini³; Rajan Kulkarni¹; Tzung Hsiai¹; Arjun Deb¹; Bin Zhou²; Haruko Nakano¹*

¹UCLA, Los Angeles, United States; ²Einstein Medical College, New York, United States

During mammalian embryogenesis, haematopoiesis occurs transiently in multiple waves in several anatomical sites. The functional role of local transient haematopoiesis during embryonic development has remained an unanswered question, given the relatively low contribution of these transient haematopoietic cells to the embryonic circulation. Here, we show that haemogenic endocardium plays a critical role in valve remodeling as a source of cardiac tissue macrophages. Haemogenic endocardial cells are enriched in the outflow and atrioventricular cushion mesenchyme at E9.5-11.5. This spatiotemporal distribution coincides with the first step of valvulogenesis, which is the mesenchymal transition of endocardial cells. Genetic lineage tracing with the endocardial specific Nfatc1-Cre mouse revealed that haemogenic endocardium gives rise to the majority of the tissue macrophages in the endocardial cushions. Characterization by surface marker analyses and gene expression profiling revealed that endocardially-derived cardiac tissue macrophages (EcTMs) predominantly play a phagocytic/antigen presenting role. Specific ablation of EcTMs caused animals to develop severe malformation of
the valves, even though the total number of cardiac tissue macrophages was compensated by recruitment of CCR2+ monocyte-derived macrophages. These results suggest that haemogenic endocardial cells give rise to a novel subset of cardiac tissue macrophages that are indispensable for the formation of valves. Our study demonstrates that local transient haemogenic activity is critical for the local tissue remodeling essential for heart valve formation.

2022 – LYMPHOMYELOID SPLIT MEDIATED BY INTERACTION-DEPENDENT TRANSCRIPTION FACTOR CHOREOGRAPHY IN PRO-T CELLS

Ellen Rothenberg1; Hiroyuki Hosokawa2; Xun Wang3; Jonas Ungerbäck2
1Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, United States; 2California Institute of Technology, Pasadena, United States; 3California Institute of Technology, Pasadena, United States

The cells that enter the thymus to become programmed into the T-lymphoid lineage initially express a multipotent progenitor gene profile and can still generate dendritic cells, granulocytes, and/or macrophages as well as T cells throughout multiple rounds of cell division in the thymus. The cells only become committed to the T-cell fate after sustained Notch signaling results in a gene network shift that causes downregulation and eventual epigenetic silencing of stem/progenitor genes. Despite detailed molecular characterization of this commitment process, it has been more challenging to account for the developmental timing of T-cell specific gene expression and cis-regulatory element activation in terms of transcription factor expression levels. There is a temporal offset, often measured in days, between the stages when pivotal factors like E2A and Runx1 first become active in the nucleus and the stages when their targets can actually respond to them. We report that their activity is constrained not only by epigenetic thresholds to site access but also by the protein-protein interaction dynamics of these factors with developmentally distinct protein complexes. Genome-wide, facultative interactions with partners including Lyl1 and PU.1 strongly influence site binding preferences of co-expressed factors in the cells. For example, although its direct binding effect on local genes is preponderantly activating, PU.1 is also shown to exert site-specific negative regulatory effects “at a distance”, at sites where it does not itself bind the DNA, through its potency in partner factor "theft". The striking shifts in global factor occupancy patterns that result from inter-complex competition provide an unexpectedly strong element in the dynamic control of hematopoietic fate determination.

2024 – EPIGENETIC REGULATION OF HEMATOPOIETIC STEM CELL DEVELOPMENT

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

The hematopoietic stem cell (HSC) system is a paradigm for stem cell research. HSCs are a population of multipotent cells that can self-renew and differentiate into all blood lineages. HSC development must be tightly controlled, which involves a complex of extrinsic signaling and intrinsic factors. Using the zebrafish and mouse models to study regulation of HSCs in vivo has greatly facilitated our understanding of HSC biology in vertebrates. I will talk about our recent progress on HSC development, with particular focus on how epigenetic factors control hemogenic endothelium-derived HSC specification. These new findings may help to design new strategies for the generation and/or expansion of transplantable and functional HSCs in vitro. (Ref: Zhang et al, Nature in press)
Late-breaking Abstracts – Posters

3264 – MICRO-VESICLES DERIVED FROM HUMAN BONE MARROW MESENCHYMAL STEM CELLS BLOCKADE PROLIFERATION OF AML-M3 CELL HL60 AND PATIENT SAMPLES

Mahnoosh Abbaszade dibavar¹; Masoud Soleimani²
¹Department of hematology and blood banking, Faculty of medical sciences, Tehran, Iran, Tehran, Iran; ²Tarbiat Modares University, Tehran, Iran

Introduction: Nowadays, one of the most prevalent forms of AML is AML-M3 which has been addressed by arsenic trioxide (ATO) and/or all-trans retinoic acid (ATRA). Although, ATRA and ATO are broadly administrated, they have dreadful side effects. Furthermore, long exposure to ATRA not only raised in chemo resistance, also lead to recurrence of the disease within three months. Altogether, there is a need for effective treatments to address chemo resistance in the APL. Recent studies considered micro-vesicles as a potential therapeutic agent. Microvesicle capacity to alter the behavior of cells is one of the most controversial issues. As point of view, micro-environment of bone marrow, normal and leukemic cells have interchangeable interaction through micro-vesicles, so micro-vesicles derived from human bone marrow mesenchymal stem cells (hBM-MSCs) might affect leukemic cells. In this study, we investigated the effect of micro-vesicles on survival of the APL cell line HL60.

Method: Microvesicles were isolated from BM-MSCs by ultracentrifugation and were added to HL60 cell line and cells of patient samples. After 7 days, cell count, cell viability by MTT assay and qPCR for BAX gene expression were performed.

Results: for the first time, we demonstrated the effect of micro-vesicles derived from human bone marrow mesenchymal stem cells on proliferation and apoptosis of HL60 cell line. This study shows, proliferation and cell growth dramatically hindered and induction of apoptosis increased in HL60 cells via staggering elevation of micro-vesicle drug doses. Interestingly patient samples mimics HL60 behavior in vitro. Anti-apoptotic genes expression dramatically decreased, and apoptotic genes show significant rising.

Conclusion: Above all, these data suggest that micro-vesicles derived from hBM-MSCs have potential capacity to act as the effective therapeutic agent in treatment of APL. However, there is a need for further studies and illustration of micro-vesicles-mediated cell death mechanisms.

3265 – DECREASED CLONOGENIC CAPACITY AND FREQUENCY OF MESENCHYMAL STROMAL CELLS DERIVED FROM ACUTE MYELOID LEUKEMIA PATIENTS ARE NOT RELATED TO AGING.

Alicia Aguilar-Navarro¹; Dafne Campa-Monroy²; Monica Reynoso¹; Eduardo Terreros-Muñoz³; Carlos Hernández-Pérez⁴; Zaida Borrego-González⁴; Luis Meillón³; Berenice Meza-León³; Ricardo Esquivel-Gómez¹; JJ Montesinos-Montesinos⁵; H Mayani⁵; Eugenia Flores-Figueroa¹
¹Microenvironment and Niche Laboratory, Mexican Institute of Social Security, Mexico City, Mexico; ²Mexican Institute of Social Security, Mexico City, Mexico; ³Microenvironment and Niche laboratory, Mexican Institute of Social Security, Mexico city, Mexico; ⁴Oncology Research Unit, Mexico City, Mexico; ⁵Oncology Research Unit, Mexico city, Mexico

Mesenchymal Stromal Cells (MSCs) are a key part hematopoietic stem/progenitor cells niche in both normal and leukemic bone marrow. The solely disruption of MSC can lead to myeloproliferation and transformation of normal hematopoietic cells. MSC from Acute Myeloid Leukemia (AML) patients harbor genetic alterations and have functional deficiencies. Given the fact that the median age at diagnosis of leukemia is 66 years old and decreased function of MSC has been reported as a result of aging, the aim of this study was to compare the clonogenic capacity and frequency of MSC derived from AML patients with MSC derived from age-paired healthy donors, in order to elucidate if those abnormalities are related to normal aging or a direct effect of the blasts. Bone marrow
samples from healthy patients were obtained from adults undergoing hip replacement surgery (n=34) and bone marrow aspirates from AML patients (n=13) from untreated patients. Samples were divided into four age groups: 25-45, 55-65, 66-75 and 80-85. CFU-F method was used to assess clonogenic capacity of MSC. MSC frequency was analyzed by flow cytometry using the following markers: CD45 and NGFR. Clonogenic capacity of MSC derived from healthy patients decreased with aging (P=0.02). AML-derived MSC showed a decreased in CFU-F number compared to their healthy counterparts in all four groups (P=0.02) but this was not associated to aging. MSC CD45-NGFR+ frequency was also decreased in AML samples compared to healthy patients (P=0.006), condition that was also independent of aging. In order to find what factor could be accounting to this decrease in both frequency and clonogenic capacity, we compared MSC CD45-NGFR+ and CFU-F number within AML samples with normal karyotype against those with chromosomal aberrations, finding that neither frequency or CFU-F number could be attributable to cytogenetic modifications. The decreased frequency and clonogenic capacity of MSC derived from AML patients is not related to aging or the blasts karyotype. Our results suggest that AML blasts directly inhibit the growth of MSC and altered their function. Conacyt FC 2015-2 Project 1341. Programa de Cooperacion Internacional. IMSS.

3266 – TARGETING MATERNAL EMBRYONIC LEUCINE-ZIPPER KINASE (MELK) IN ACUTE LYMPHOBLASTIC LEUKEMIA
Houda Alachkar1,2,3; Martin Mutonga4; Amanda de Albuquerque5; Rucha Deo6; Gregory Malnassy 5; Yusuke Nakamura5; Wendy Stock5
1USC, Los Angeles, United States; 2Los Angeles, Los Angeles, United States; 3California, Los Angeles, United States; 4North Western, Chicago, United States; 5University of Chicago, Chicago, United States; 6University of Southern California, Los Angeles, United States

Unlike the high cure rates of children with acute lymphoblastic leukemia (ALL); that of adults is still lagging behind and better therapies are needed. Maternal embryonic leucine-zipper kinase (MELK), is aberrantly upregulated in several types of malignancies and is implicated in formation and maintenance of cancer stem cells. We recently showed that MELK is a therapeutic target in acute myeloid leukemia (AML). Targeting MELK inhibited FOXM1 and its downstream target genes in AML cells. FOXM1, a substrate of MELK, was recently found to be a therapeutic target in B-cell lineage ALL (B-ALL). In addition, β-Catenin is required for T-cell leukemia initiation. Importantly, a crosstalk between FOXM1 and the β-catenin has been implicated in cancer stem cells. Thus, we hypothesized that MELK may act as a therapeutic target in ALL via regulating FOXM1/β-Catenin signaling pathways. In a panel of 14 ALL cell lines, MELK and FOXM1 expression were significantly higher in B-ALL and T-ALL compared to that in mononuclear cells obtained from healthy donors. MELK knockdown significantly decreased cell viability (40-70 %, P < 0.05) in B and T-ALL cells; this effect was also associated with induced apoptosis as measured by flow cytometry (Annexin V), and the increase of cleaved caspase 3 measured by western blot. Similarly, OTS167, a potent inhibitor of MELK exhibited various cytotoxic activities in both B and T leukemia cells. We found the IC50 of OTS167 ranged from 20-60nM; we also found a significant increase in apoptosis (P < 0.05) in B- and T-ALL cell lines. Mechanistically, MELK inhibition resulted in a decrease of FOXM1 protein levels as early as 3 hours following treatment. Furthermore, MELK inhibitor reduced P-GSK3β but not total GSK3β, and also decreased β-Catenin. In conclusion, MELK is highly expressed in ALL and present a novel therapeutic target likely via modulating FOXM1/β-Catenin activity. OTS167 is currently undergoing Phase I/II in Patients with Refractory or Relapsed AML, ALL and advanced other hematologic malignancies. Therefore, further functional and mechanistic studies are highly needed to complement the clinical investigation of this therapeutic approach.

3267 – SETD1A PROTECTS HSCS FROM DNA DAMAGE-INDUCED FUNCTIONAL DECREASE IN VIVO
Kathrin Arndt; Claudia Wasikow; Andrea Kranz
Technical University Dresden, Dresden, Germany

The regenerative capacity of hematopoietic stem cells (HSCs) is limited by the accumulation of DNA damage and very few protective pathways are known. SETD1A is one of six histone methyltransferases placing methylation marks on histone 3 lysine 4 (H3K4), an epigenetic signature related to active transcription. Spatiotemporally controlled deletion of Setd1a in adult HSCs is compatible with adult life and has no effect on the maintenance of phenotypic HSCs in the bone marrow. However, SETD1A-deficient stem cells lose their transcriptional cellular identity and acquire a short-term HSC profile. Loss of transcriptional HSC identity is accompanied by the complete
absence of stem cell function under stress situations in situ and after transplantation. Mechanistically, SETD1A is dispensable for cellular survival but essential for HSC proliferation and differentiation by regulating the expression of genes important for virtually every DNA damage detection and repair mechanism. Finally, we show that SETD1A is a key mediator for the protection of HSCs from activation-induced attrition in response to inflammatory stimulation. In summary, we have uncovered an unexpected role for SETD1A in protecting HSCs from DNA-damage-mediated loss of function in vivo.

3268 – SPLICEOSOMAL RNA MODIFICATIONS IN NORMAL AND MALIGNANT HEMATOPOIESIS

Giulia Beneventi; Maciej Ciesla; Roberto Munita Robert; Cristian Bellodi
Division of Molecular Hematology, Lund University, Sweden, Lund, Sweden

Splicing is a central step controlling the coding capacity of the genome during fundamental cellular processes such as development and tumorigenesis. The spliceosome, a dynamic macromolecular machinery consisting of a spliceosomal RNA (snRNA) backbone bound to several protein cofactors, performs the splicing reaction. Despite findings that splicing is commonly altered in many cancers, the mechanisms whereby oncoproteins hijack the spliceosome to promote tumorigenesis remain poorly understood. A central step during spliceosome biogenesis is the chemical modification of snRNA with pseudouridine (Ψ) and 2'-O-methyl ribose (2'OMe) residues. These post-transcriptional ‘marks’ are introduced within key functional regions of the snRNA by evolutionarily conserved small ribonucleoproteins (SNPs) particles. Interestingly, small RNPs impairments are common in hematological cancers, frequently characterized by hematopoietic stem cell (HSC) and splicing dysfunctions. Hence, an important outstanding question is the role of snRNA modification defects in leukemogenesis. Here, we comprehensively investigated how major oncogenic pathways ‘rewrite’ the landscape of snRNA modifications to alter splicing during the early steps of malignant transformation in primary human cells. Furthermore, we extended these findings in vivo and developed a novel mouse model to inactivate the Ψ small RNP machinery directly in the hematopoietic compartment. Using this genetic tool, we are currently delineating the mechanistic basis by which snRNA pseudouridylation and splicing impairments impact normal and malignant hematopoiesis. Altogether, results from these studies will define an unparalleled RNA modification-driven program that governs splicing dynamics during leukemogenesis with promisingly broad implications for hematological cancers characterized by HSC dysfunction.

3269 – MEMORY OF CELL DIVISIONS DIRECTS THE CONTINUOUS PROCESS OF PRIMITIVE HEMATOPOIETIC LINEAGE COMMITMENT.

Jeffrey Bernitz1; Dmitrii Scherbinin2; Andreia Gomes3; Yuan Ye3; Michael Daniel3; Avinash Waghray3; Huen-Suk Kim1; Dmitri Papatsenko2; Kateri Moore1
1Icahn School of Medicine at Mount Sinai, New York, United States; 2Skolkovo Institute of Science and Technology, Moscow, Russia

Hematopoietic stem cells (HSCs) exist in a dormant state, and cycle infrequently over time. Label-retaining studies show that HSCs progressively lose regenerative potency as they increase their divisional history, indicating HSCs maintain a memory of their cell divisions. How this information is encoded is unclear. To understand how these memories are stored, we performed RNA sequencing on HSC populations differing only in their cumulative cell divisions over time. Comparison of four HSC populations distinguished by increasing divisional histories found 2715 genes differentially expressed, even though all populations were sorted from the same HSC gate. These genes either increase or decrease their expression as cells divide over time. Genes that increase their expression are enriched for factors associated with lineage commitment, and are regulated by FoxM1, E2Fs, and Myc—transcription factors (TFs) that drive cell cycle progression. Additionally, mature lineage genes have transcripts expressed at very low levels in HSCs that progressively increase in expression with cell divisions indicating that proliferation may be driving lineage priming. In contrast, many genes that decrease with divisions are associated with an HSC signature, and are enriched for targets of the Polycomb Repressive Complex 2 (PRC2). Interestingly, the PRC2 catalytic subunits Ezh1 and Ezh2 have opposite expression patterns, where Ezh1 is the primary catalytic subunit in dormant cells, and Ezh2 is progressively activated with divisions. The expression of global HSC H3K27me3 target genes also increase or decrease with progressive divisions, suggesting histone mark regulation is also divisional history dependent. Finally, we show that Ezh1 represses cell cycle genes and activates genes important for HSC identity to maintain dormancy, while Ezh2 represses HSC signature genes. Our study reveals a
positive feedback loop where cell division drives the expression of cell cycle TFs, which further promote cell divisions, drive lineage priming, and activate Ezh2 expression. Ezh2 then accumulates and targets HSC signature genes for repression to consolidate information on divisional history into memory.

3270 – EFFICACY OF COLLAGEN BINDING ASSAY IN VON WILLEBRAND DISEASE DIAGNOSIS
Mengyuan Ding
Soochow University, Suzhou, China (People's Republic)

BACKGROUND: von Willebrand disease (VWD) is a congenital bleeding disorder caused by either a quantitative or a qualitative defect in von Willebrand factor (VWF). Measurement of VWF multimer distribution is critical for diagnosis of variant VWD, but the typical measurement by gel electrophoresis is technically difficult and time consuming. Currently, collagen-binding assays (CBA) based on the agarose gel electrophoresis technique that measure the interaction of VWF and collagen are an alternative analytic procedure for VWF measurement. The aim of this study was to evaluate the efficacy of CBA in VWD diagnosis.

METHODS: A total of 21 cases of VWD patients and 9 healthy control subjects were included in this study. The serum were collected from these subjects and analyzed by CBA, VWF antigen (VWF:Ag) assay and ristocetin cofactor activity (RCo) assay. The results from these three assays were compared and between-assay correlation coefficient was calculated.

RESULTS: A statistically significant reduction of serum VWF level was found in patients with VWD compared to the healthy controls by CBA, VWF:Ag assay and RCo assay (all the p < 0.01) and the mean ratio detected by CBA, VWF:Ag assay and RCo assay for those individuals with abnormal multimer distribution was 11.05%, 6.31% and 6.07% respectively. The correlation coefficient of CBA and VWF:Ag assay was 0.975, whereas the correlation coefficient of CBA and RCo assay was 0.897, suggesting a good correlation between CBA and VWF:Ag Assay or RCo assay.

CONCLUSIONS: Our results indicated that similar efficacy but simple procedure of CBA compared to VWF:Ag assay or RCo assay, which could make it possible to determine the serum VWF level in a quick manner and may provide a diagnosis tool for those patients with VWD.

3271 – STRESS HEMATOPOIESIS AND ITS POTENTIAL CANCEROUS IMPACT
Roi Gazit1,2,3
1Ben-Gurion University of the Negev, Beer-Sheva, Israel; 2Beer-Sheva, Beer-Sheva, Israel; 3Select, Beer-Sheva, Israel

Hematopoietic Stem Cells (HSCs) possess the unique attributes of self-renewal and multipotency, which enable healthy generation of blood and immune cells. HSCs are also the likely cell of origin for hematologic malignancy if perturbed. Stress-hematopoiesis, such as chronic infection and inflammation, is known to induce a pre-malignant state and increase the risk of leukemia. However, the mechanistic route via which stress mediates transformation is unclear and there are no well-defined models with which to interrogate this phenomenon. Recently developed model of repeated induction of interferons can cause chronic stress-hematopoiesis with impact on HSCs. Using multiple HSC-markers that allow us to prospectively isolate and molecularly characterize the stem- and progenitors-populations after acute and chronic stress. Combining genomic- and transcriptome-analysis is aiming to reveal molecular mechanisms of aberrations in HSCs. Studying both bulk-population as well as single-cell data is leading to realization of the general changes and possible clonal-heterogeneity of HSCs. We further aim to functionally determine pathways that lead to leukemia initiation and progression upon stress hematopoiesis. Revealing how stress-hematopoiesis is skewing HSCs towards malignancy will suggest new ways to modulate pre-malignant state and hopefully minimize the risk of blood cancer.
3273 – VEGF PLAYS AN ESSENTIAL ROLE IN THE INDUCTION AND THE RESOLUTION OF SPLENIC EXTRAMEDULLARY HEMATOPOIESIS DURING PREGNANCY

Miriam Grunewald¹; Husne Shariffe³; Rosanne Turner⁴; Ingeborg Bajema⁴; Shay Porat⁵; Gail Amir⁶; Eli Keshet⁶
¹Hadassah MEdical School, The Hebrew University, Jerusalem, Israel; ²Hadassah MEditional School, The Hebrew UNiveristy, Jerusalem, Israel; ³University of Leiden, LEiden, Netherlands; ⁴University of Leiden, Leiden, Netherlands; ⁵Hadassah Hospital, Jerusalem, Israel; ⁶Hadassah Medical School, THe Hebrew university, Jerusalem, Israel

Adult hematopoietic stem cells (HSCs) reside primarily in the bone marrow of mammals in which a specialized microenvironment ensures their proper functioning. However, upon a variety of hematopoietic stresses, HSCs can egress from the BM to the circulation and initiate hematopoiesis in remote organs. While medullary hematopoiesis is under extensive investigation, little is known about extramedullary hematopoiesis (EMH). In particular, the factors that naturally stimulate and resolve EMH are not fully identified. Here, we show that VEGF, a pleiotropic factor that orchestrates neovascularization and plays also multiple non-vascular roles in organ homeostasis, is essential for the transient EMH triggered during pregnancy in mice. Increasing systemic levels of VEGF correlate with increasing splenic EMH and VEGF titration by endogenous soluble VEGF receptor 1 (SFLT) produced by the placenta correlates with the resolution of EMH around birth. Using a transgenic system, we show that premature sequestration of VEGF during pregnancy reduces significantly HSCs number and erythropoiesis in the spleen. Interestingly, we show for the first time evidence of EMH in spleen sections of pregnant woman, which is much attenuated in spleen obtained from pregnant women suffering from preeclampsia, a condition where levels of endogenous SFLT are abnormally elevated.

Altogether, our results demonstrate an essential role of VEGF in the transient induction of extramedullary hematopoiesis in mice.

3274 – DIGOXIN INHIBITS CELL METASTASIS IN HEPATOCELLULAR CARCINOMA: PERCEPTIONS OF ANNEXIN A2(ANXA2) SHRNA

Wei-Ju Huang³; Yung-Ming Jeng³; Hung-Pin Hsu⁴
³Department of Oral Hygiene, Hsin-Sheng College of Medical Care and Management, Taoyuan, Taiwan., NEW TAIPEI CITY, Taiwan (Republic of China); ⁴Graduate Institute of Pathology, College of Medicine, National Taiwan University, Taiwan, Taiwan., NEW TAIPEI CITY, Taiwan (Republic of China); ⁵National Taiwan University Hospital, Taipei, Taiwan, TAIPEI, Taiwan (Republic of China); ⁶Taipei City Hospital, Taipei, Taiwan., NEW TAIPEI CITY, Taiwan (Republic of China)

Background: Digoxin (DG) a cardiac glycosides and Na(+)/K(+) ATPase inhibitor, was a traditional drug for various heart disease therapy, like atrial fibrillation, atrial flutter and sometimes heart failure. Evidence showed that cardiac glycosides affected several pathways for tumor formation and had potential for cancer therapy. Annexin 2 (ANXA2), a calcium-dependent phospholipid-binding protein, is involved in diverse cellular processes such as cell motility, linkage of membrane-associated protein complexes to the actin cytoskeleton, endocytosis, fibrinolysis, ion channel formation, and cell matrix interactions. ANXA2 overexpression is important to maintain the malignancy of cancer cells. The aim of this study is to find the inhibition of DG on cell metastasis in hepatocellular carcinoma (HCC) by knockdown of ANXA2 RNA.

Material and Methods: The shRNA is transfected in 293T breast cancer cell line, shANXA2-6322 and shANXA2-9717, to product the virus cultured suspension, which can make the several HCC cancer cell lines to express ANXA2 or not in virus infection, and they were used to treated with DG in effective dose. Cells apoptosis was observed by MTT assay, cells containing transforming oncogenes grown in focus-forming assay.

Results: We seed the shANXA2-6322 and shANXA2-9717 cell line in the uncoating transwell plate to observe the cell migration and invasion. The number in all cell lines were similar in unwipe. After wipe, the cell migration and invasion were decreased in shANXA2-6322 and shANXA2-9717 cell line comparing to the HA22T and RFPi group. It proved that in liver cancer, ANXA2 is the key factor in cell migration and invasion. Prospectively, the shANXA2-6322 and shANXA2-9717 cell survival rate were significantly decreased in MTT assay. Finally, we use different doses of DG to treat HA22T, RFPi, shANXA2-6322 and shANXA2-9717 cell line. After treating with DG, the decrease in cell migration and invasion were stronger especially in DG at dose of 0.1uM.

International Society for Experimental Hematology (ISEH)
www.iseh.org
HEMATOPOIETIC STEM CELLS FROM FETAL LIVER AND BONE MARROW PRESENT DUALITY OF RESPONSE TO PROLIFERATION

Satish Khurana1; Atreya Biswas2; Selen Abanuz3; Javed Manesia3; Sarah Schouteden3; Albert Santa-Maria4; Joerg Huelsken5; Adam Lacy-Hulbert6; Catherine Verfaillie7

1Indian Institute of Science Education and Research Thiruvananthapuram, Thiruvananthapuram, India; 2IISER TVM, Thiruvananthapuram, India; 3KU Leuven, Leuven, Belgium; 4University of Fribourg, Fribourg, Switzerland; 5EPFL, Lausanne, Switzerland; 6Benaroya Research Institute, Seattle, United States; 7KU Leuven, Leuven, India

Stem cell function and ageing is regulated by intrinsic as well as extrinsic factors. Adult stem cells are maintained in the state of quiescence, which maintains their stemness and protects them from physiological stress. During development, on the other hand, these stem cells proliferate symmetrically to create a pool, sufficient for entire lifetime. We hypothesized that the physiological and genetic differences could be important in identifying molecules that regulate proliferation state of the stem cells. We performed RNASeq analysis of HSCs and their
niche components across developmental stages and identified outside-in integrin signaling as an important regulator of HSC quiescence in adult bone marrow (Khurana S. et al. in Nat. Comm. 2016). Periostin (Postn) via binding with Integrin-αvβ3 (Itgav-b3) regulated their proliferation affecting their stemness. Both, Postn-/− and Vav-iCre;Itgavfl/fl mice showed functional decline of HSCs and a phenotype similar to pre-mature ageing of the hematopoietic system. Postn inhibited culture-induced proliferation of WT but not Itgav-/− HSCs, which proliferated faster compared with Itgav+/+ cells. While interruption of Postn-Itgav interaction led to the loss of quiescence and hence exhaustion of adult HSCs, it led to efficient expansion of HSCs in the developing fetal liver (FL). We observed increased frequency of long-term repopulating HSCs derived from Postn-/− E14.5 FL, which engrafted equally well upon transplantation. We identified metabolic differences between the adult and FL derived HSCs, which could explain this duality of response towards the loss of Postn-Itgav interaction (Manesia J. et al. in Stem Cell Research 2015). Overall, we demonstrate the importance of ligand-mediated integrin signaling in proliferation and function of HSCs during fetal and adult stages.

3276 – EXPLORING THE ABILITY OF THE FETAL RNA BINDING PROTEIN LIN28B TO ALLEVIATE PROLIFERATION INDUCED HEMATOPOIETIC STEM CELL EXHAUSTION AT THE CLONAL LEVEL
Trine Kristiansen1; Alexander Doyle2; Ewa Ohlsson3; Linda Starnes3; Andres Lopez-Contreras4; David Bryder5; Jeremy Daniel6; Joan Yuan5
1Lund University, Medical Faculty, Institution for Laboratory Medicine, Division of Molecular Hematology, Sweden, Lund, Sweden; 2Lund University, Medical Faculty, Institution for Laboratory Medicine, Division of Molecular Hematology, Sweden, Lund, Sweden; 3Chromatin Structure and Function Group, The Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, Copenhagen, Denmark; 4Department of Cellular and Molecular Medicine, Center for Chromosome Stability and Center for Healthy Aging, Panum Institute, University of Copenhagen, Copenhagen, Denmark, Copenhagen, Denmark; 5Lund University, Medical Faculty, Institution for Laboratory Medicine, Division of Molecular Hematology, Sweden StemTherapy, Lund, Sweden

Hematopoietic stem cells (HSCs) undergo a functional switch in neonatal mice upon achievement of equilibrium in the blood and immune systems. This switch is hallmarked by a decrease in self-renewing divisions and entry into quiescence, with subsequent proliferation being increasingly associated with functional HSC exhaustion. This developmental switch coincides with decreased expression of Lin28b, a fetal specific RNA binding protein capable of reinitiating fetal-like hematopoiesis when ectopically expressed in adult bone marrow. The basis for Lin28b induced hematopoietic rejuvenation could hold important clues to age dependent changes in hematopoiesis. However the underlying mechanisms remain poorly understood to date. Here, we use cellular barcoding to quantify the impact of Lin28b on the behavior of single HSC clones and their response to replicative stress.

We demonstrate that ectopic expression of Lin28b in adult HSCs recapitulates fetal-like HSC self-renewal, resulting in prolonged maintenance of functional HSC clones following transplantation into irradiated recipients. Furthermore, Lin28b delays the relative decline in lymphoid output at the clonal level, and thereby the onset of a well-established myeloid bias associated with age. These findings establish clonally defined criteria for fetal-like HSC function and demonstrate a role for Lin28b in alleviating functional exhaustion associated with HSC transplantation. Unlike the adult, fetal HSCs undergo rapid proliferation without overt signs of HSC exhaustion. Thus, we propose that Lin28b actively safeguards stem cell fitness and alleviates stress in the face of increased proliferation and metabolism to meet the extraordinary growth demands during intrauterine hematopoiesis. Current efforts are aimed at assessing the potential contribution of replication stress alleviation in Lin28b induced fetal-like HSC clonal behavior and combines genetic perturbations in the replication stress signaling pathway with cellular barcoding.

International Society for Experimental Hematology (ISEH)
www.iseh.org
3277 – MODELLING ETV6-RUNX1 ASSOCIATED CHILDHOOD ACUTE LYMPHOBLASTIC LEUKAEMIA IN INDUCED PLURIPOTENT STEM CELLS

Emma Laycock1; Simon Richardson1; Charlotte Böiers2; Tariq Enver1
1UCL Cancer Institute, London, United Kingdom; 2Lund University, London, United Kingdom

Childhood acute lymphoblastic leukemia (cALL) is clinically distinct from that in adults with a higher incidence, better prognosis and distinct mutational spectrum. cALL is often initiated in utero indicating that first-hit mutations may arise in progenitors in developmentally restricted haematopoietic sites. We use human induced pluripotent stem cells (iPSCs) to model the effect of the ALL-initiating oncogene ETV6-RUNX1 in an in vitro B cell differentiation system that mimics the early embryonic development of human haematopoietic cells. Genome engineering iPSCs to constitutively express ETV6-RUNX1 results in a block in B cell development at the level of a novel lympho-myeloid progenitor that is abundant in the early fetal liver. The few ETV6-RUNX1 B cells that emerge aberrantly co-express B and myeloid signatures and retain myeloid potential. In addition, in a competitive in vitro assay, primitive ETV6-RUNX1 CD34+CD45+CD19- progenitors outcompeted wild type. We propose that ETV6-RUNX1 specifically impacts the cell fate decision of the novel lympho-myeloid progenitor, providing a rationale for ETV6-RUNX1 ALL as a disease of childhood.

3278 – HETEROGENEOUS RESPONSES OF HEMATOPOIETIC STEM CELLS TO INFLAMMATORY STIMULI ARE ALTERED WITH AGE

Matí Mann1; Arnav Mehta2; Carl Boer3; Monika Kowalczyk4; Kevin Lee5; Aviv Regev6; David Baltimore6
1Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, United States; 2Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, United States; 3Broad Institute of MIT and Harvard, Cambridge, United States; 4Broad Institute of MIT and Harvard, Cambridge, United States; 5Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, United States; 6Broad Institute of MIT and Harvard. Howard Hughes Medical Institute, Koch Institute of Integrative Cancer Biology, Department of Biology, Massachusetts Institute of Technology, Cambridge, United States

Long-term hematopoietic stem cells (LT-HSCs) maintain hematopoietic output throughout an animal’s lifespan. However, with age the balance is disrupted and LT-HSCs produce a myeloid-biased output. This, in turn, may result in poor immune responses to infectious challenge and the development of myeloid leukemias. We show that young and aged LT-HSCs respond differently to inflammatory stress, such that aged LT-HSCs produce a cell-intrinsic, myeloid-biased expression program. Using single-cell RNA-seq (scRNA-seq), we identify a myeloid-biased LT-HSC subset (mLT-HSCs) that is much more common amongst aged LT-HSCs and is uniquely primed to respond to acute inflammatory challenge. We predict several transcription factors to regulate differentially expressed genes between mLT-HSCs and other LT-HSC subsets. Among these, we show that Klf5, Ikaros1 and Stat3 play an important role in age-related inflammatory myeloid bias. These factors may regulate myeloid versus lymphoid balance with age, and mitigate the long-term deleterious effects of inflammation that lead to hematopoietic pathologies.

3279 – MESENCHYMAL STROMAL CELLS FROM DOG AND HUMAN BONE MARROW SHARE THE SAME MARKERS, DISTRIBUTION, FREQUENCY AND CELLULAR FUNCTIONALITY

Asela Berenice Meza León1; Alicia Aguilar Navarro2; Beremiz Sánchez Salazar3; Álvaro Zugarazo4; Samuel Medina4; Ariel Meza5; Ricardo Esquivel6; Juan José Montesinos6; Eugenia Flores Figueroa6
1Universidad Nacional Autónoma de México, Ciudad de México, Mexico; 2UNAM, Ciudad de México, Mexico; 3UVM, Ciudad de México, Mexico; 4None, Ciudad de México, Mexico; 5IMSS, Ciudad de México, Mexico

The study of Mesenchymal Stromal Cells (MSC) represents a new promissory avenue to look for new prognostic factors in hematological diseases. MSC are a key component of the niche in normal and leukemic hematopoiesis. Hence, it is important to have in vivo models for the study of MSC in normal and leukemic settings. Dogs are a good model to study human hematopoiesis because their share similar biological characteristics; however, in dogs, there are no standardized methods to identify and assess MSC in situ. The aim of this study is to establish a standardized method to identify, quantitate and study their cellular functionality MSC from Dog bone marrow. In order to identify markers of MSC in dog bone marrow, 9 samples from dogs were collected during orthopedic surgery (3 months to 8 years old) we assessed human (CD271) MSC marker by immunohistochemistry. We

International Society for Experimental Hematology (ISEH)

www.iseh.org
evaluated their distribution and frequency. Images were digitized and quantitated with an image analysis software (CellProfiler). Functional assays were carried out through colony assays (CFU-F) and in vitro cultures to assess their differentiation (adipogenic, osteogenic and chondrogenic). Protocol was approved by Subcommittee Institutional Animal Care Experimentation and Ethics committee in health research R-2012-785-092.

CD271 was expressed in cells with reticular morphology. CD271+ cells localized in four areas in bone marrow: perivascular, perimegacariocytic, peritrabecular and parenchyma. The frequency of this population was 55.16 ± 6.15 which is similar to human samples (n=8) 61.13 ± 13.86 (p = 0.10). Functionally, MSC are able to form colonies at a frequency of 5.53 ± 4.30 colonies/100,000 cells whereas in human was 1.99 ± 1.38 colonies/100,000 cells (p= 0.035), besides MSC have the capacity to differentiate towards adipocytes, osteoblasts and chondrocytes. CD271 is a suitable marker to assess the frequency and distribution of dog MSC in situ. Dog MSC shared the same frequency, distribution and cellular functionality than their human counterpart. Dogs represent a good model to study MSC in normal settings. We need to study leukemic dog bone marrow in order to compare it with their human counterpart.

3280 – SALL4-TRANSDUCED EXPANDED UMBILICAL CORD DERIVED CD133+ CELLS MAINTAINED STEMNESS GENE EXPRESSION

Majid Mossahebi-mohammad1; masoud soleimani 2; Amir Atashi 3; Saeid Kaviani 4
1Department of hematology and blood banking, Faculty of medical sciences, Tehran, Iran; 2Tehran; 3Tata Modares University, Tehran, Iran; 4Shahrroud, Iran, Shahrroud, Iran; 5Tarbiat Modares University, tehran, Iran

Abstract

Hematopoietic stem cells (HSCs) were characterized by self-renewal and multilineage potential. Umbilical cord blood-derived (UCB) as an alternative source of HSCs is widely used in stem cell transplantation. The main limitation in using UCB for transplantation especially in adults is low cell dose. To improve this hindrance besides using double dose UCB, ex vivo expansion is the most important way to increase cell number for transplantation. CD133, as the most primitive marker, shows important physiological role in maintenance and expansion of HSCs. SALL4 plays crucial role in the development and maintaining the pluripotency and self-renewal ability of embryonic stem cells (ESCs) as well as HSCs expansion, normal hematopoiesis and hematological malignancies. In current study, SALL4 transduced expanded CD133+ were assessed for expression of pluripotency genes including Oct-4, Sox-2, klf-4, c-Myc, PS3 and Bmi1 using quantitative real time PCR (qRT-PCR). Moreover, Karyotyping analysis was performed to assess any chromosomal instability and I leukemogenesis potential of SALL4 in hematopoietic expanded cells after 7 days. Obtained results demonstrated that SALL-4 transduced cells showed expression of stemness genes at higher levels in SALL-4 transduced group compared to cytokine expanded hematopoietic cells and hESCs group as positive control. Our results illustrated that SALL4 could act as a positive factor for the expansion of CD133+ derived UCB cells besides maintaining stemness potential and any numerical and structural chromosomal aberrations.

Keywords: Hematopoietic stem cells expansion, Karyotyping, Stemness genes, SALL4

3281 – ELUCIDATING THE AGE INDUCED HEMATOPOIETIC CELL-INTRINSIC AND EXTRINSIC MECHANISMS IN MYELOPROLIFERATIVE NEOPLASM INITIATION AND PROGRESSION

Tata Nageswara Rao1; Tata Nageswara Rao2; Nils Hansen3; Simón Méndez-Ferrer4; Radek Skoda1
1Experimental Hematology, Department of Biomedicine, University Hospital Basel, Basel, Switzerland, Basel, Switzerland; 2Wellcome Trust-Medical Research Council Cambridge Stem Cell Institute and Department of Haematology, University of Cambridge, and National Health Service Blood and Transplant, Cambridge Biomedical Campus, Cambridge, UK, United Kingdom

The number of detectable somatic mutations increase with age, but this increase is surpassed by the rise in the incidence of cancer in older people. The underlying mechanisms for this disparity remain to be elucidated. Nonetheless, the prevalent occurrence of such clonal events in aged individuals brings up the question, which age-associated factors contribute to initiate hematologic malignancies and what are the rate limiting steps attributable for age-induced malignancies? Myeloproliferative neoplasm (MPN) is an ideal malignancy model disease to study clonal hematopoiesis, disease evolution during aging because the majority of the relevant mutations (such as JAK2 V617F) are catalogued, the disease evolves and progresses slowly allowing the collection of serial samples, and inducible transgenic mouse models for the disease have been established. We hypothesize that aging induced

International Society for Experimental Hematology (ISEH)

www.iseh.org
alterations provides a context that facilitates acquisition of additional mutations and/or confer selection for pre-malignant clones, and that activation of mutant JAK2 further augments these changes for increased MPN incidence in aged individuals.

To identify age associated factors and the rate limiting steps attributable for initiation and progression of MPN, we studied young and aged inducible transgenic mouse models of MPN carrying a JAK2-V617F mutation. Integrated omics analysis was conducted on HSPCs from young and aged MPN mice. Our results suggest that age related changes in expression patterns resembling MPN can be found in aged wildtype mice. Bone marrow transplantations with naturally aged donors and recipients revealed hematopoietic cell-intrinsic and cell-extrinsic (niche) contributions in MPN progression. Ongoing mechanistic studies investigate the relative contributions and collaborations of age-associated cell intrinsic and extrinsic changes in HSCPs and BM niche in the course and severity of MPN. Our study provides novel molecular and cellular mechanisms underlying increased incidence of MPN manifestation in old age. This knowledge ultimately will help to define novel strategies to delay or target the onset of MPN in an aging individual.

3282 – VE-CADHERIN CONTROLS BONE MARROW PERMEABILITY AND HOMING OF HEMATOPOIETIC STEM CELLS.
Martijn Nolte1; Timo Rademakers3; Marieke Goedhart1; Mark Hoogenboezem1; Maryna Samus2; Stefan Butz2; Stephan Huveneers2; Carlijn Voermans1; Dietmar Vestweber1; Jaap van Buul1
1Sanquin, Amsterdam, Netherlands; 2Max Planck Institute for Molecular Biomedicine, Muenster, Germany; 3AMC, Amsterdam, Netherlands; 4Max Planck Institute for Molecular Biomedicine, Muenster, Netherlands

The vasculature of the bone marrow (BM) plays an important role in generating HSC niches, but it is also crucial for adequate engraftment of HSCs upon transplantation. Despite these essential functions of blood vessels in the BM, we know surprisingly little of how they control local permeability and HSC migration. To better comprehend the contribution of BM endothelial cells to these processes, we employed in vivo and ex vivo imaging of the BM vasculature in murine femurs by multiphoton microscopy. We discovered that the vascular endothelial-specific barrier regulator VE-cadherin plays an eminent role in controlling vascular integrity in the BM. Blocking VE-cadherin function drastically promoted vascular leakage in BM, but not in lung, under resting conditions and further enhanced BM permeability upon sub-lethal body irradiation. Reversely, locking junctional VE-cadherin (using VE-cadherin-alpha-catenin knock-in mice) increased vascular integrity, even after irradiation. VE-cadherin-mediated permeability also influenced HSC migration, as homing of HSCs to BM of sub-lethally irradiated mice was strongly enhanced when VE-cadherin was blocked. Interestingly, HSC migration was not inhibited in VE-cadherin-alpha-catenin knock-in mice. This led to the hypothesis that HSCs can use either trans- or paracellular migration, depending on how tight the endothelial cell junctions are, as the former is junction-independent. When testing this in vitro, we indeed observed that human CD34+ HSPCs cross a tight monolayer of BM endothelial cells mostly in a transcellular manner. However, when VE-cadherin function was blocked, the morality of the HSPCs switched to paracellular transmigration. This indicates that HSPCs can use both routes of transendothelial migration to enter the BM and that the chosen path depends on the tightness of the endothelial junctions.

In summary, we show that VE-cadherin is crucial for controlling BM vascular homeostasis and regulating HSC homing after transplantation. This study thus identifies VE-cadherin as a new target to increase the efficiency of HSC transplantation.

3283 – DYNAMIC REGULATION OF RUNX1 AT THE ONSET OF DEFINITIVE HEMATOPOIESIS
Dominic Owens
University of Oxford, Oxford, United Kingdom

Hematopoietic stem cells (HSCs) have been used in medicine for several decades. However, the difficulty of generating, maintaining or expanding them in culture has made it difficult to advance HSC-based therapies further. In the embryo, HSCs first arise from a subset of the dorsal aorta endothelium, the so-called hemogenic endothelium (HE), through a process known as endothelial-to-hematopoietic transition (EHT). The transcription factor Runx1 is a critical regulator of EHT and in its absence cells are blocked at the HE stage. We have identified Runx1 cis-regulatory elements to facilitate elucidating the trans-acting factors that mediate Runx1 expression and thus hematopoietic specification and EHT. In our ongoing work, we identified all Runx1 cis-interactions in 3D, using
next generation chromosome conformation capture (NG Capture-C) and confirmed enhancers previously identified in our lab as bona fide cis-interacting elements. To establish the Runx1 enhancer-specific activity within discreet cell populations of EHT, we generated and characterized novel transgenic enhancer-reporter mouse lines. Interestingly, two of the novel transgenic enhancer-reporters mark distinct cell stages during EHT: one specifically marks HE, while the other marks hematopoietic progenitors but not the HSC lineage. Both are in contrast to the Runx1 +23 enhancer-reporter, which marks all cells undergoing EHT, including HE and hematopoietic stem and progenitor cells. In our ongoing work we are deleting Runx1 enhancers from the endogenous locus in mouse ES cells, to assess which of these are necessary for Runx1 expression and begin to identify the upstream regulatory pathways that mediate Runx1 expression at distinct stages of EHT. These studies aim to provide a detailed understanding of the dynamic regulation of Runx1 expression at the onset of hematopoiesis and the factors and signals involved in establishing the HSC lineage. A better understanding of how HSCs are generated in mouse ontogeny is expected to inform the development of culture protocols to generate these cells in vitro.

3284 – MOLECULAR DETERMINANTS FOR POLYCOMB GROUP PROTEIN YY1 CONTROL OF HEMATOPOIETIC STEM CELL QUESCENCE

Xuan Pan1,2; Courtney Hong3; Guangyao Kong4; Zhanping Lu1; Anna Assumpção5; Irene Ong3; Emery Bresnick1; Jing Zhang6

1Department of Medical Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, Wisconsin, Madison, United States; 2Carbone Cancer Center, UW-Madison Blood Research Program, Madison, United States; 3University of Wisconsin-Madison, Madison, United States; 4National Local Joint Engineering Research Center of Biodiagnostics and Biotherapy, The Second Affiliated Hospital of Xi’an Jiaotong University, Xi’an, China (People’s Republic)

Yin Yang 1 (YY1) is a ubiquitous transcription factor and mammalian Polycomb Group Protein (PcG) with important functions to regulate embryonic development, lineage differentiation and cell proliferation. YY1 mediates stable PcG-dependent transcriptional repression via recruitment of PcG proteins that catalyze histone modifications. Many questions remain unanswered regarding how cell- and tissue-specific regulation is achieved by PcG proteins. Herein, we conditionally deleted Yy1 in mouse hematopoietic cells (Yy1f/f Mx1-Cre) and demonstrated that Yy1 deficiency decreases hematopoietic stem cell (HSC) long-term repopulating activity. Moreover, ectopic YY1 expression expands HSCs. Yy1−/− mice were pancytopenic with a significantly shorter median survival time compared with wild-type control mice. YY1 deficiency deregulated the genetic network governing HSC proliferation and impaired Stem Cell Factor (SCF)/c-Kit signaling, thus disrupting mechanisms conferring HSC quiescence. As our prior study demonstrated that YY1 PcG function is required for Ig chain rearrangement in early B cell development, we evaluated the role of YY1PcG function in HSC self-renewal and addressed whether YY1 PcG function is context-dependent in adult hematopoiesis. Ectopic expression of a YY1 mutant lacking the PcG function (YY1REPO) in YY1−/− mice rescued SCF/c-Kit signaling, HSC quiescence and maintained the capacity of HSC self-renewal. Thus, YY1 regulates adult HSC quiescence in a PcG-independent manner. These results reveal a unique mechanism for how a ubiquitously expressed transcriptional repressor mediates lineage-specific functions to control adult hematopoiesis. Currently, we are testing how YY1 differentially controls HSCs and B cells via PcG-independent and –dependent mechanisms respectively.

3285 – CICLOPIROX ETHANOLAMINE IS A NOVEL MODIFIER OF HUMAN HEMATOPOIETIC STEM CELL EX VIVO EXPANSION

Mehrnaz Safae Talkhoncheh1; Fredrik EK2; Aurelie Baudet1; Agatheeswaran Subramaniam3; Christine Karlsson1; Roger Olsson2; Jonas Larsson2

1Molecular Medicine and Gene Therapy, Lund Stem Cell Center, Lund University, Lund, Sweden; 2Department of Experimental Medical Science, Lund University, Lund, Sweden

Application of small molecule screening to modulate stem cells has emerged as a useful tool for identification of new compounds with ability to expand hematopoietic stem cells (HSCs). To search broadly for potential modifiers of ex vivo HSC expansion, we screened 500 small molecules for the phenotypic expansion of human cord blood derived CD34+CD38− cells following a 6-day ex vivo culture. From the screen, several candidate hits were selected and subjected to a validation experiments from which we selected four top candidates. One of these, Ciclopirox

International Society for Experimental Hematology (ISEH)

www.iseh.org
ethanolamine (CPX), had previously not been implicated in HSC expansion. CPX is known as an antifungal agent and iron chelator. We found that CB cells cultured with CPX had a 3-fold increase in CD34+90+ cell number compared to DMSO control. Interestingly, the total cell count was not different, suggesting a specific increase in CD34+CD90+ cell number rather than an overall higher proliferation rate. To further test the functional capacity of cells cultured with CPX, we transplanted cultured equivalents of 30,000 CB CD34+ cells (cultured with or without CPX) into sub lethally irradiated NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ (NSG) mice. Human chimerism in peripheral blood was determined 16 weeks later. Mice transplanted with CPX cultured cells showed higher human CD45 engraftment 16 weeks post transplant compared to control cells (33.2±6.7% vs 14.6±5% p=0.04). The engrafted cells contributed to both myeloid and lymphoid lineages. This shows that CPX enhances the long-term engraftment capacity of ex vivo cultured HSCs and suggests that it should be considered in stem cell expansion protocols, either alone or in combination with other molecules. Indeed, we found that CPX synergized with the aryl hydrocarbon antagonist SR1 in promoting expansion of CD34+90+ cells. Molecular analysis of CPX treated cells showed decreased levels of reactive oxygen species as well as reduced mitochondrial activity. Currently we are addressing the mechanism of CPX treatment in more detail and we are comparing it further with other recently defined modifiers of HSC expansion.

3286 – OXYGEN SENSING ENZYME JMJD6 IS A REGULATOR OF HSC SELF-RENEWAL AND A TUMOR SUPPRESSOR IN ACUTE MYELOID LEUKAEMIA
Catarina Sepulveda1; Amélie Guitart2; Milica Vukovic3; Theano Panagopoulou4; Lewis Allen1; Hannah Lawson1; Andreas Lengelling5; Kamil Kranc1
1MRC Centre for Regenerative Medicine, University of Edinburgh, UK, Edinburgh, United Kingdom; 22Biotherapy of Genetic diseases, Inflammatory disorders and Cancer, Université de Bordeaux, France, Bordeaux, France; 31MRC Centre for Regenerative Medicine, University of Edinburgh, UK, Edinburgh, United Kingdom; 43Beckman Research Institute of City of Hope, Duarte, CA, USA, Los Angeles, United States; 54The Roslin Institute, Edinburgh, UK, Edinburgh, United Kingdom

The regulation of haematopoietic stem and progenitor cells (HSPCs) is crucial to ensure a normal multilineage haematopoiesis. Acute myeloid leukaemia (AML) is a clonal disorder of HSPCs, which acquire mutations and form treatment-resistant leukaemic stem cells (LSCs) that fuel the disease. To design improved therapies that eradicate LSCs, it is essential to fully understand the processes which promote and suppress leukaemogenesis. Emerging studies have described that key oncometabolites inhibit 2-oxoglutarate dependent dioxygenases (2-OGDO), a family of enzymes that has been reported to play an important role in leukaemogenesis. Here we investigated the impact of one of these 2-OGDO, Jmjd6 (a jumonji protein which regulates RNA splicing) on normal haematopoiesis and pathogenesis of AML.

We found that genetic deletion of Jmjd6 specifically from the haematopoietic system perturbed the homeostasis of the HSPC pool and resulted in a myeloid bias. Serial transplantation experiments demonstrated that HSCs lacking Jmjd6 did not efficiently reconstitute multilineage haematopoiesis in primary recipients and completely failed to repopulate secondary recipients. Thus Jmjd6 is essential for HSC self-renewal and maintenance. We have also assessed the impact of Jmjd6 deletion in the context of inflammatory response and recovery from treatment with a myelotoxic agent treatment, which revealed that Jmjd6 is a positive regulator of HSC homeostasis, and recovery from cytotoxic stress.

Given the role of Jmjd6 in regulating RNA splicing, a process frequently affected in blood malignancies, we investigated the role of Jmjd6 in AML initiation and propagation in a Meis1/Hoxa9-mediated murine model. We found that Jmjd6 deletion resulted in a rapid onset of AML and Jmjd6-deficient LSCs rapidly propagated the disease. Thus we unraveled a new biological function for Jmjd6 as a tumor suppressor in AML.

Taken together, our findings offer important novel insights into the biological functions of Jmjd6 and pave the way for further studies to discover the mechanism of action of this complex enzyme.
EPCR MARKS THE LONG-TERM REPOPULATING ACTIVITY OF CULTURED HUMAN CORD BLOOD CELLS
Agatheeswaran Subramaniam1;2; Mehrnaz Safaee Talkhoncheh1; Jonas Larsson3

Umbilical cord blood (UCB) is a promising source of hematopoietic stem cells (HSCs) for cell therapy of malignant hematological disorders. Due to the limited number of HSCs from a single UCB unit, in-vitro expansion may be necessary to achieve a transplantable cell dose. The cell surface marker combination of CD34+CD38-CD45RA-CD90+CD49f+ marks long-term HSCs from fresh UCB. However, except for CD34 and CD90, the expression patterns of these markers are not specifically associated with stem cell activity following culture. Identification of additional markers to more precisely label HSCs in culture would greatly facilitate the development and evaluation of conditions for HSC expansion. Endothelial protein C receptor (EPCR), also known as CD201, is encoded by the PROCR gene. EPCR is the cell surface receptor for activated protein C and essential for anti-coagulation and to mediate anti-inflammatory signals. Previous studies in mice established EPCR as a highly specific HSC marker. In humans, gene expression profiling shows that EPCR is preferentially expressed in HSCs compared to multipotent progenitors (MPP) from both UCB and fetal liver. Using flow cytometry, we observed distinct EPCR expression in HSC enriched fractions from UCB and fetal liver and we are currently exploring the functional properties of these EPCR positive populations. We further detected EPCR expression in cultured UCB derived HSCs within a fraction of the CD34+CD90+ population which contains all HSC activity. Interestingly, we found that the fraction of EPCR+ cells increased under conditions that favor HSC expansion, such as the addition of UM171 (3.167±0.9025% in DMSO VS 41,73±1,79% in UM171), suggesting that EPCR may serve as a marker for HSCs in culture. To address this further, we cultured UCB HSCs for 6 days and then transplanted the CD34+CD90+EPCR+ and CD34+CD90+EPCR- populations into sublethally irradiated NSG mice. Mice receiving the EPCR+ population showed significantly higher engraftment of human cells after 16 weeks (63,92±7,052% vs 14,11±8,669%). Altogether our study suggests that EPCR can be a useful marker to track human HSCs in culture.

EPIGENETIC ANALYSIS OF HUMANIZED MICE REFLECTS HEMATOPOIETIC DEVELOPMENT AND ACCELERATED AGING
Wolfgang Wagner1; Joana Frobel1; Susann Rahmig2; Julia Franzen1; Claudia Waskow2
1RWTH Aachen University Medical School, Aachen, Germany; 2Technical University Dresden, Dresden, Germany

Transplantation of human hematopoietic stem cells (HSCs) into immunodeficient mice is a valuable technique to gain insight into stemness and hematopoietic development. However, it is yet unclear if transplanted human cells recapitulate epigenetic changes of normal hematopoietic development. Furthermore, age-associated DNA methylation (DNAm) changes are acquired faster in mice than man, corresponding to their shorter life-expectancy, and therefore epigenetic aging might be accelerated in the xenogenic transplantation setting. In this study, we analyzed DNAm patterns of stably engrafted human hematopoietic cells in KIT-deficient NOD/SCID II2rg−/−KitW41/W41 (NSGW41) mice. Five NSGW41 mice were transplanted with CD34+ cells from human umbilical cord blood (CB). Bone marrow was harvested after 19 weeks, sorted for human CD45+ cells, and global DNAm profiles were analyzed with Illumina 450k BeadChips. Flow cytometric analysis of sorted CD45+ cells demonstrated that 71% (+/- 3%) are CD19+, whereas 22% (+/- 6%) express myeloid surface markers. In comparison to DNAm profiles of various mature human hematopoietic subsets the transplanted cells remained epigenetically closely related to CD34+ CB cells, whereas deconvolution of cell-type specific DNAm patterns overall recapitulated immunophenotypic analysis. Consistent with the high CD19+ content of engrafted cells, promoter regions of various B cell associated genes, such as CD19, were hypomethylated. We filtered for CG dinucleotides (CpGs) that distinguish B cells from other mature hematopoietic cell types and found that in humanized mice the DNAm patterns were closely related to those of immature B cells, corresponding to phenotypic analysis. Furthermore, different epigenetic aging signatures consistently indicated that 19 weeks after transplantation epigenetic aging is moderately but significantly accelerated in mice (1 to 5 years). Taken together, hematopoietic development in humanized mice is recapitulated epigenetically. Notably, epigenetic aging seems to be moderately accelerated in mice, indicating that the xenogenic microenvironment impacts on the aging process.
Definitive hematopoiesis is featured by the generation of hematopoietic stem cells (HSCs), the process of which involves various precursors and multiple locations simultaneously. Up to date, none of known markers including CD41 and CD45 can specifically and continuously recognize the entire process of definitive hematopoiesis, which hampers the precise understanding of the HSC formation during development. Here, we reported that primitive erythroid progenitors lacked CD43 expression, in contrast to definitive hematopoietic progenitor cells, all of which resided within CD43+ population. Further, CD43 expression marked the first cohort of HSCs in E10.5 aorta gonad mesonephros (AGM) region and all of other locations including head, yolk sac, and placenta later, and further enriched emerging HSCs within CD34+c-Kit+ population. In fetal liver, expanding HSCs also expressed high level CD43. CD43+ cells in AGM region lacked endothelial potential in vitro, indicative of its segregation from endothelial lineage. Thus, we re-defined endothelial cells as CD31+CD41-CD43-CD45-Ter119- population in E10-E11 AGM. Of note, we successfully recapitulated endothelium-to-HSC transition by an ex vivo induction, and uncovered a previously unknown CD41-CD45-CD43+ HSC-competent population. Together, our findings identify CD43 as a first faithful surface marker for definitive hematopoiesis throughout mouse development.

WIP1 PHOSPHATASE IS REQUIRED FOR HEMATOPOIETIC STEM CELL MATURATION IN MOUSE EMBRYOS
Xiaona Zheng1; Wenyan He1; Yu Lan2; Bing Liu1
1Affiliated Hospital of Academy of Military Medical Sciences, Beijing, China (People's Republic); 2Jinan University, Guangzhou, China (People's Republic)

Hematopoietic stem cells (HSCs) are generated through a process of multistep maturation and expansion in mouse embryo, and each stage has varied cellular phenotype and some specific molecular markers. Although HSCs development process is principally documented to be governed by complex mechanisms, the regulation of HSCs maturation process remains poorly defined. Here, we find that Wip1 deletion leads to reduced number and defective function of HSCs and hematopoietic progenitor cells (HPCs) in E12.5 fetal liver. Most strikingly, the adult-reconstituting HSCs with self-renewal capacity are nearly absent in the E11.5-12.5 Wip1-/ AGM as revealed by direct transplantation assay. However, initial organ culture results in dramatic recovery of HSC potential in Wip1-/AGM, suggesting blocked maturation of pre-HSCs in vivo. Furthermore, quantitative analysis showed that the phenotypic defined T1 (CD31+CD45-CD41low-c-Kit+CD201high) and T2 (CD31+CD45+c-Kit+CD201high) pre-HSCs inordinately decreased in E11 Wip1-/AGM region, especially T2 pre-HSCs showed lowered donor chimerism in transplantation assay after co-cultured with OP9-DL1 stromal cells. Consistently, incubation of T1 and T2 pre-HSCs from E11.0 wild type AGM with CCT007093 (the specific inhibitor of Wip1 phosphatase), abrogated and severely impaired HSCs formation, respectively. Taken together, these findings position phosphatase Wip1 is indispensable for Pre-HSC/HSC maturation in the AGM region, suggesting quite differential regulatory mechanisms on HSC generation among distinct developmental stages.