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摘要手册

Abstract Manual

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Abstract

A1

Severe Fanconi Anemia phenotype in novel Fancd2 Knockout mice

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Fanconi anemia (FA) is a genetic disorder associated with congenital developmental defects, bone marrow failure (BMF) and predisposition to cancers.In this report, we generated a novel Fancd2 knockout mouse model in C57BL/6 background with CRISPR-Cas9. Two independent F0 founder lines with 1bp and 7bp deletion in the exon5 were identified and separately crossed to produce homozygous *Fancd2*$^{-/-}$ mice. Both *Fancd2*$^{-/-}$ mice lines show complete loss of FANCD2 protein and exhibited similar but more severe FA phenotype comparing to previously reported *Fancd2*$^{-/-}$ mouse model. This *Fancd2*$^{-/-}$ miceexhibitedhigh embryoniclethalityand pre-mature mortality rate. Thehomozygous *Fancd2*$^{-/-}$ mice was only around 5% of all the born pups present at the time of genotyping (10 day prenatal) versus the expected 25%Mendelian ratio. Also, about 30% of the*Fancd2*$^{-/-}$mice die before 3 weeks age and another 20% die before 8 weeks old. The *Fancd2*$^{-/-}$ mice reach adulthood exhibited overall smaller size, single or both side microphthalmia or abnormal eye development. The testes and ovary organ from *Fancd2*$^{-/-}$ mice were smaller in size and under developed. The mononuclear bone marrow cells isolated from *Fancd2*$^{-/-}$ mice exhibited hypersensitivity to MMC and X-ray irradiation treatment.Specifically,*Fancd2*$^{-/-}$mice show mild but significantly hematology abnormal. Also, hematopoietic stem and progenitor cells (HSPCs, LK, LSK, SLAM cells) from*Fancd2*$^{-/-}$were significantly decreased in number. Multilineage reconstitution experiment indicated dramatically reduced function of*Fancd2*$^{-/-}$HSCs. We also observed aberrant cell cycle and H2AX status of*Fancd2*$^{-/-}$HSPCs. In this context, Fancd2 play an important role in mouse early development and thisFancd2mouse model may be a useful tool for studying Fanconi Anemia pathophysiology.

Keywords: Fanconi Anemia, Fancd2, mouse model

A2

Prediction of Competing Endogenous RNA Coexpression Network as Prognostic Markers in AML

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Acute myeloid leukemia (AML) is an aggressive hematological malignancy which is characterized by abnormal proliferation and differentiation of myeloid progenitor cells. Targeting therapy of aberrant signaling pathways in AML has been increasingly recognized as a promising approach to eradicate AML. Alterations which occur in genes with important roles in regulating chromatin in hematopoietic progenitors, or exert a role in altering epigenetic pattern contribute to hematological pathogenesis, and could represent attractive targets in AML treatment. In recent years, the regulatory network composed of long non-coding RNAs (lncRNAs), microRNAs (miRNAs) and messenger RNAs (mRNAs), has gained a great interest in the study of molecular biological mechanisms of AML occurrence and progression. However, studies on AML are limited, and there is still a lack of comprehensive analysis of lncRNA-miRNA-mRNA ceRNA regulatory network of AML based on high-throughput sequencing and large-scale sample size. In this study, we obtained RNA-seq expression data and compared the mRNAs and lncRNAs expression profiles between 407 normal whole bloods (GTEx dataset) and 151 bone marrows of AML (TCGA dataset) by edgeR package of R platform. Ensemble ID of lncRNAs and mRNAs that were not included in the gencode database were excluded. All q values used false discovery rate (FDR) to correct the statistical significance of the multiple test. Fold changes (log2 absolute) $\geqq 2$ and FDR < 0.01 were considered significantly. ClusterProfiler was used for GO functional enrichment and KEGG Gene Set Enrichment Analysis. WGCNA was applied to enrich mRNA-cluster and lncRNA-cluster with traits of all samples. WGCNA uses adjacency to measure the similarity between normal and AML set genes in the network, which was calculated based on the correlation coefficient. Following, we used miRcode and StarBase to predict interactions between lncRNA and miRNAs. MiRTarBase, miRDB and TargetScan databases were applied to predict miRNA targeted mRNAs. We chose the overlapped targeted mRNAs by analyzing the predicted targeted mRNAs, WGCNA-most related mRNAs, as well as the significantly up-regulated genes and down-regulated genes by edgeR. Lastly, we got 111 up-regulated targeted genes and 9 down-regulated targeted genes. To identify independent factors associated with survival in AML, a univariate Cox proportional hazard regression analysis was conducted to clarify the association of the expression levels of 120 genes with overall survival (OS). 22 genes were obtained by the standard of P value<0.05 and gene ID <15000. Then, the above-mentioned 22 genes were brought into further multivariate Cox proportional hazard regression analysis. Based on the above analysis, we obtained a set of mRNAs highly related to the survival and prognosis of AML. We set up a survival model for 3-year OS: HOXA9+INSR+KRIT1+MYB+SPRY2+UBE2V1+WEE1+ZNF711. Finally, we identified 108

lncRNAs, 10 miRNAs and 8 mRNAs to build the lncRNA-miRNA-mRNA ceRNA network, which might act as biomarkers to predict the survival of AML patients. ceRNA networks could open an important stratum of study on gene regulation, development and prognosis of AML.

Keywords: ceRNA, AML

B1

New megakaryocyte progenitors phenotypically similar to hematopoietic stem cells

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Content: Recent purification studies have suggested that megakaryocytes develop closely related to hematopoietic stem cells (HSCs). Multipotent progenitors 2 (MPP2) has been reported as megakaryocyte progenitor population. This study was performed to illustrate early megakaryopoiesis pathway in murine bone marrow. Methods: HSC1 ($CD150^+CD41^-CD34^-LSK$ cells) and HSC2 ($CD150^-CD41^-CD34^-LSK$) in bone marrow of C57BL/6 mice were further divided into $CD48^-CD201^-$, $CD48^-CD201^+$ and $CD48^+$ fractions based on CD48 and CD201 expression. Transplantation was performed to examine the reconstitution potential for each fraction. Single-cell colony assay were performed for each fraction in the presence of SCF, TPO, IL-3, and EPO. MPP2 ($Lin^-Sca\text{-}1^+c\text{-}Kit^+Flk2^-CD150^+CD48^+$) and MkP ($Lin^-Sca\text{-}1^-c\text{-}Kit^+CD150^+CD41^+$) were also examined. Results: Myeloid-biased long-term HSCs were mainly detected in $CD48^-CD201^+$ HSC1, but were also detectable in $CD48^-CD201^-$ HSC1. Lymphoid-biased short-term HSCs were mainly detected in $CD48^-CD201^+$ HSC2, but were also detectable in $CD48^-CD201^-$ HSC2. Reconstitution activity was not detected in $CD48^+$ fractions of HSC1 and HSC2. $CD48^-CD201^+$ fractions of HSC1 and HSC2 were enriched in nmEM (n, neutrophil; m, macrophage; E, erythroblast; M, megakaryocyte) colony-forming cells. $CD48^-CD201^-$HSC1 and $CD48^+$HSC1 fractions were enriched in megakaryocyte colony-forming cells. $CD48^-CD201^-$HSC1 didn't overlap with MPP2, and size of megakaryocyte colony derived from this fraction were larger than size of those derived from MPP2 and MkP. Pair-daughter cell assay of $CD48^-CD201^+$ HSC1 showed a very low frequency of megakaryocyte and nmEM combination (1/32 pairs). Conclusion: A new megakaryocyte progenitor population was identified, which was phenotypically related to LT-HSC population. This new population was suggested to be downstream of HSCs and upstream of MPP2 in megakaryocyte differentiation pathway.

Keywords: HSC, Megakaryocyte, colony-forming assay

B2

PDK1 regulates definitive HSCs via the FOXO pathway during murine fetal liver hematopoiesis

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Content: PDK1 (phosphoinositide dependent kinase-1) plays an important regulatory role in B cells, T cells and platelets. Less is known about how PDK1 acts in hematopoietic stem cells (HSCs), especially in the fetal liver (FL) during embryonic hematopoiesis, as the FL is the primary fetal hematopoietic organ and the main site of HSC expansion and differentiation. Methods: Transplantation assay Cells from the fetal hematopoietic organs were transplanted together with 1×105 nucleated BM cells of CD45.1 mice to promote short-term survival. Female CD45.1 mice were exposed to a split dose of 9 Gy γ-irradiation (60Co). Peripheral blood (PB) of the recipients was collected at the indicated time points. Greater than 10% of CD45.2 cells determined by FACS was considered as successful reconstitution. HSC homing assay 2-3ee fetal liver cells from wild type (WT) or Vav-Cre;PDK1f/f embryos stained by CFSE were injected into CD45.2 mouse. Recipients were sacrificed to get bone marrow cells and spleen cells 17 hours later since fetal liver cells were injected. FACS was performed to examine the CFSE positive bone marrow cells or spleen cells. Colony-forming cell (CFC) assay The sorted LT-HSC, ST-HSCs and MPP from WT and Vav-Cre;PDK1f/f mice were cultured in MethoCult GF M3434 medium (Stem Cell Technologies) containing various cytokines to support the hematopoietic progenitors. Colonies were counted after 3–14 days of culture according to the manufacturer's instructions. mRNA sequencing analysis RNA samples were prepared by the SMARTer protocol (Shanghai Biotechnology Corporation). Reads were aligned to the mouse genome GRCm38.*P*4 (mm10), the generated counts were normalized for mRNA abundance, and differential expression analysis was performed using edgeR. GSEA was performed using the pre-ranked option in combination with log fold change values of each comparison calculated by edgeR. Results: Here, we deleted the PDK1 gene in hematopoietic cells by crossing Vav-Cre transgenic mice with PDK1f/f mice. Using a transplantation assay, we found that HSCs from the E15.5 FL of Vav-Cre;PDK1f/f embryos are severely impaired compared when compared with HSCs from PDK1f/f or PDK1f/+ FLs. Additionally, we found that there were more FL HSCs in an apoptotic state and active cell cycle in PDK1-deficient embryos than in control embryos. By comparing the expression profiles of FL-derived LSKs in Vav-Cre;PDK1f/f embryos to the controls, we found that the BH3-only protein PUMA and the cyclin family proteins were expressed higher in the Vav-Cre;PDK1f/f group, which may account for the increased apoptosis and activated cell cycle in the deficient HSCs. Furthermore, we demonstrated that the expression of FoxO3a was higher in PDK1-deficient LSKs, indicating that the Akt-FoxO3a-PUMA axis may participate in regulating LSKs apoptosis in the E15.5 FL. In contrast, FoxO1 expression was lower in PDK1-deficient LSK cells, suggesting that Akt-FoxO1-CCND may regulate the HSC cell cycle.

Conclusion: Taken together, our findings support a critical role for PDK1 in maintaining FL hematopoiesis via regulating apoptosis and cell cycle of definitive hematopoiesis by the Akt-FOXO signaling pathways.

Keywords: HSC, PDK1, AKT

C1

Acute Myeloid Leukemia Cells Overexpressing P2X7 Have Increased Level of Leukemia Stem Cells

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Content: *P*2X7 is the latest cloned member of the *P*2X family ligand-gated ion channel receptors. High level expression of *P*2X7 is reported in various malignant cells. High level expression of *P*2X7 was also detected in different leukemia patients, especially in relapsed cases. However, the role of *P*2X7 in leukemia was poorly understood. In the present study, we studied the effects of *P*2X7 receptor in acute myeloid leukemia mouse model. Methods: We established MLL-AF9 induced AML mouse model with high level expression of *P*2X7 (MA9-*P*2X7). The sorted leukemia cells were assessed *in vitro* colony-forming ability assay and *in viivo* limiting dilution transplantation experiments. Meanwhile, the leukemia cells were clustered by marker c-Kit, and the survival was recorded after different populations were transplanted into recipient mice. Furthermore, multiple administrations of Ara-C to leukemia mice was performed. Results: MA9-*P*2X7 mice had shorter survival time than control mice without high level of *P*2X7 (MA9). The *in vitro* colony-forming ability assay showed that MA9-*P*2X7 cells formed more colonies, especially type B and C colonies, than MA9 cells. Limiting dilution transplantation experiments showed that the frequency of leukemia stem cells (LSCs) was estimated at 1/2088 in MA9 cells and 1/288 in MA9-*P*2X7 cells, suggesting a 7.25-folds increase of functional LSCs in MA9-*P*2X7. We also detected the expression of c-Kit, and the results showed that the majority of MA9-*P*2X7 cells were c-Kit$^+$, whereas MA9 cells have two populations and more than half of them were c-Kit$^-$. Though mice transplanted with MA9 c-Kit$^+$ cells had a shorter survival time than those transplanted with MA9 c-Kit$^-$ cells, those transplanted with MA9-*P*2X7 cells had the shortest survival time. Upon treatment with Ara-C, though MA9-*P*2X7 cells were more sensitive to Ara-C, the mice relapsed quickly and had shorter survival time. Conclusion: Our results suggested that leukemia cells overexpressing *P*2X7 possessed the characteristics of higher LSCs frequency, which contributed to more malignant phenotype of leukemia.

Keywords: P2X7, MLL-AF9

C2

Inflammation-associated cytokines IGFBP1 and RANTES impair the megakaryocytic potential of HSCs in PT patients after allo-HSCT

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Prolonged isolated thrombocytopenia (PT) isa severe complication in patients after allogeneic hematopoietic stemcell transplantation (allo-HSCT). Whetherthe megakaryoctic potentialof hematopoietic stemcells(HSCs) inbone marrow isintact and what factorsdrive thepathological processof PTremain elusive. A retrospective study in patients (n=285) receiving HSC transplantation revealed that the occurrence of PT was approximately 8% and the number of platelets and megakaryocytes in PT patients is much lower compared to the control. To test whether the deficiency of thrombopoiesis was caused by the activities of HSCs, the megakaryocytic differentiation potential of HSCs before or after transplantation was assessed. Interestingly, a substantial decrease of megakaryocytic differentiation was observed two weeks after transplantation of HSCs in all of the allo-HSCT recipients. However, four weeks after transplantation, the ability of HSCs to generate $CD41^{+}CD42b^{+}$megakaryocytes in SPE patients recovered to the same level as those of HSCs before implantation. In contrast, HSCs derived from PT patients throughout the post-implantation period exhibited poor survival and failed to differentiate properly. A protein array analysis demonstrated that multiple inflammation-associated cytokines were elevated in allo-HSCT recipients with PT. Among them, IGFBP1 and RANTES were found to significantly suppress the proliferation and megakaryocytic differentiation of HSCs *in vitro*. Our results suggested that the occurrence of PT might be attributed, at least partially, to the damage to HSC function caused by inflammation-associated cytokines after HSC transplantation. These findings shed light on the mechanism underlying HSC megakaryocytic differentiation in PT patients and might provide potential new strategies for treating PT patients after HSC transplantation.

Keywords: megakaryopoiesis , allo-HSCT, PT

C3

Jagged-1 secreted by stromal cells mediated the different response of HSCs and HPCs to leukemic condition

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Leukemia is the competition failure between normal and abnormal hematopoiesis. Our previous work found that in Notch1-induced murine T-ALL, normal HSCs were preserved in part because of increased mitotic quiescence of HSCs and resulted exhaustion of HPCs proliferation. The differential expression of Hes1 between HSCs and HPCs resulted in the distinct responses of these cells to the leukemic condition. But the mechanism of how leukemic environment affect the expression of Hes1 in normal HSC is still unknown. To elucidate the mechanism, upstream factors of Hes1 especially the cell surface receptor is detected. Also, the ligand and its origin cells are determined. The results find that Notch1 signaling pathway in normal HSCs is activated while it's silenced in normal HPCs. The adjacent cells around HSCs are stromal cells which secrete Jagged-1. Depletion of Jagged-1 will disrupt the balance between self-renew and proliferation of HSCs leading to their exhaustion. Our study will provide new ideas for the pathogenesis of leukemia, and provide a theoretical basis for the treatment of leukemia.

Keywords: Jagged-1, stromal cells, leukemia

C4

IL-22 accelerates regeneration of thymus via STAT3/Mcl-1 in murine allogeneic hematopoietic cell transplants

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Pretransplant conditioning of allogeneic hematopoietic cell transplants (allo-HCT) severely damagesthymic epithelial cells (TECs) of recipient thymus resulting in poor posttransplant immune recovery. IL-22 mediates recovery of TECs *via* a pro-regenerative effect but how this occurs is unknown. We found donor T-cell-derived IL-22 improved thymus recovery in allograft recipients in association with increased number of TECs.This effect is blocked by ruxolitinib, a *JAK1*/*JAK2*-inhibitor. IL-22 stimulates proliferation of TECs *via* STAT3-dependent signaling. STAT3 regulates transcription of myeloid cell leukemia sequence1 (*Mcl1*) which then regulates proliferative effects of IL-22 on a murine thymic epithelial cell line (mTEC1). In addition, exogenous IL-22 reverses chronic graft-versus-host disease (cGVHD) in murine allotransplant recipients. Our data highlight the critical role of the IL-22/STAT3/Mcl-1 pathway in regeneration of TECs after allotransplants in mice.

Keywords: allogeneic hematopoietic cell transplantation, thymus, IL-22

C5

JAM3 maintains leukemia-initiating cell self-renewal through LRP5/AKT/beta-catenin/CCND1 signaling

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Leukemia-initiating cells (LICs) are responsible for the initiation, development and relapse of leukemia. The identification of novel therapeutic LIC targets is critical to curing leukemia. We herein reveal thatjunctional adhesion molecule3 (JAM3) is highly enriched in both mouse and human LICs. Leukemogenesis is almost completely abrogated upon *Jam3* deletion during serial transplantations in an MLL-AF9-induced murine acute myeloid leukemia model. In contrast, *Jam3* deletion does not affect the functions of mouse hematopoietic stem cells. Moreover, knockdown of *JAM3* leads to a dramatic decrease in the proliferation of both human leukemia cell lines and primary LICs. JAM3 directly associates with LRP5 to activate the downstream PDK1/AKT pathway, followed by the down-regulation of GSK3b and activation of b-catenin/CCND1 signaling to maintain the self-renewal ability and cell cycle entry of LICs. JAM3 may serve as a functional LIC marker and play an important role in the maintenance of LIC stemness through unexpected LRP5/PDK1/AKT/GSK3b/b-catenin/CCND1 signaling pathways but not via its canonical role in cell junctions and migration. JAM3 may be an ideal therapeutic target for the eradication of LICs without influencing normal hematopoiesis.

Keywords: JAM3, leukemia-initiating cells, self-renewal

C6

SHQ1 regulation of RNA splicing is required for T-lymphoblastic leukemia cell survival

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T-acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic malignancy with complicated heterogeneity. Although expression profiling reveals common elevated genes in distinct T-ALL subtypes, little is known about their functional role(s) and regulatory mechanism(s). We here show SHQ1, an H/ACA snoRNP assembly factor involved in snRNA pseudouridylation, is highly expressed in T-ALL. Mechanistically, oncogenic NOTCH1 directly binds to the *SHQ1* promoter and activates its transcription. *SHQ1* depletion induces T-ALL cell death *in vitro* and prolongs animal survival in murine T-ALL models. RNA-Seq reveals that *SHQ1* depletion impairs widespread RNA splicing, and *MYC* is one of the most prominently downregulated genes due to inefficient splicing. *MYC* overexpression significantly rescues T-ALL cell death resulted from *SHQ1* inactivation. We herein report a mechanism of NOTCH1-SHQ1-MYC axis in T cell leukemogenesis. These findings not only shed light on the role of SHQ1 in RNA splicing and tumorigenesis, but also provide additional insight into *MYC* regulation.

Keywords: NOTCH1, T cell leukemia, RNA splicing

C7

Spectrum of somatic mutations and their prognostic significance in adult patients with B-acute lymphoblastic leukemia

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Objective Target-specific next-generation sequencing technology was used to analyze the regions of 112 genes in adult patients with B-acute lymphoblastic leukemia (B-ALL). The influences on prognosis of different gene mutations were analyzed. Methods The study prepared DNA samples from 113 adult B-ALL patients. Target-specific next generation sequencing (NGS) approach was used to analyze the mutations of 112 genes (focused on the specific mutational hotspots) and all putative mutations were compared against multiple databases. Then the frequency spectrum and the impact on the patients' prognosis were analyzed by the putative mutations through Kaplan-Meier, and Cox regression methods. Results Of the 113 patients, 103 (92.0%) harbored at least one mutation and 29 patients (25.6%) harbored more than 3 genes mutation. The five most frequently mutated genes in B-ALL are *SF1*、*FAT1*、*MPL*、 *PTPN11*、*NRAS*. Gene mutations are different between Ph^+B-ALL and Ph^-B-ALL patients. Ph^-B-ALL patients with JAK-STAT signal pathway genes mutation, such as JAK1/JAK2 mutation showed a poor prognosis. Patients with PTPN11 mutation showed better survival. Besides, Ph^+B-ALL patients whose epigenetic modifications signaling pathways genes were affected had a worse prognosis. Conclusion Our study suggests that gene mutations exists in adult ALL patients universally, involving a variety of signaling pathways. The frequency and species are varied in different types of B-ALL. Abnormal JAK family often indicates poor prognosis. The co-occurrence of somatic mutations in adult B-ALL patients suggests the complex and unstable genetic composition.

Keywords: Acute lymphoblastic leukemia,B cells; Target-specific next-generation sequencing; Somatic mutations; Prognostic significance.

D1

Combination of SCF plus G-CSF or GM-CSF enhances proliferation of hematopoietic progenitor cells but not stem cells

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Content: Stem cell factor (SCF) directly stimulates hematopoietic stem and progenitor cells and acts synergistically with other cytokines to promote their proliferation. A significantly enhanced proliferation of committed progenitor cells was observed by using a combination of SCF + G-CSF or GM-CSF in vitro. However, whether there is also such a synergistic effect on proliferation and fate decision of hematopoietic stem cellsis poorly understood. Here our study aimed to clarify the synergistic effect of SCF plus G-CSF or GM-CSF on hematopoietic stem cells. Methods: SingleHSC1($CD201^+CD150^+CD48^-CD41^-CD34^-KSL$), HSC2 ($CD201^+CD150^-CD48^-CD41^-CD34^-KSL$), HPC1 ($CD201^+CD150^-CD48^-CD41^+CD34^-KSL$), HPC2 ($CD150^+Flt\text{-}3^-CD34^+KSL$),HPC3 ($CD150^-Flt\text{-}3^-CD34^+KSL$), and HPC4 ($CD150^-Flt\text{-}3^+CD34^+KSL$) cells were cultured in serum-free medium with cytokines for 7 days. For transplantation, cells from day 7 culture were injected into lethally irradiated mice. Results: Proliferation of HPC2, HPC3, and HPC4 cells were enhanced in culture with SCF+G-CSF, SCF+GM-CSF, and SCF+G-CSF+GM-CSF when compared with that in SCF alone. However, there was no significant increase of HSC1, HSC2, and HPC1 cells. Transplantation with 10 freshly isolated cells showed that the percentage of myeloid reconstitution was maintained for as long as 9 months after transplantation. Transplantation with cellscultured with SCF alone showed that the myeloid reconstitution was well maintained as freshly isolated cells did. However, transplantation with cellscultured with SCF+G-CSF, SCF+GM-CSF, SCF+G-CSF+GM-CSFshowed no significant enhancement in reconstitution levels. These results showed that G-CSF and GM-CSF have no effect on the fate decision of stem cells. Conclusion: SCF alone can directly maintain HSC reconstitution, and the target cells of G-CSF and GM-CSF were progenitor cells.

Keywords: hematopoietic stem cells, progenitor cells, G-CSF

D2

The role of hematopoietic stem cells in early reconstitution

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Content: Hematopoietic stem cells (HSCs) are a group of cells in the hematopoietic system with self-renewal and multi-lineage differentiation potential, which can be detected by bone marrow transplantation. HSCs can be divided into long-term HSCs (LT-HSCs) and short-term HSCs (ST-HSCs) according to the duration of hematopoietic reconstitution after transplantation. However, whether HSCs play a role in early hematopoiesis (< 1 month after transplantation) is not so clear. Methods: To address this issue, we analyzed HSCs and HPCs by early hematopoietic reconstitution and colony-forming unit-spleen (CFU-S) assays. According to the immune-phenotype, the hematopoietic stem/progenitor cells(HSPCs) were divided into six groups, HSC1 (CD201+CD150+CD48-CD41-CD34-LSK), HSC2 (CD201+CD150-CD48-CD41-CD34-LSK), HPC1 (CD201+CD150+CD48-CD41+CD34-LSK), HPC2 (CD150+Flt3-CD34+LSK), HPC3 (CD150-Flt3-CD34+LSK), and HPC4 (CD150+Flt3+CD34+LSK). 100 or 300 cells were sorted from bone marrow of B6-Ly5.1 mice, and transplanted into B6-Ly5.2 mice irradiated at the dose of 8.5 Gy without competitive cells. Peripheral blood (PB) and bone marrow (BM) cells of recipient mice were analyzed within 30 days after transplantation. The spleens of mice were examined on days 7 and 12 after transplantation. Results: Transplantation with 300 HSC1, 2, HPC1, 2, 3, 4 cells showed that donor cells could be detected in PB and BM as early as day 12 after transplantation. The percentage of chimerism in PB (mean± S.D.) were 10.1 ± 6.4%, 47.0 ± 16.8%, 36.2 ± 18.6% ,12.8 ± 25.6%, 14.8 ± 21.4%，3.6± 2.3%, respectively, for HSC1, 2, HPC1, 2, 3, 4. The percentage of chimerism in BM were 9.4 ± 13.2%，20.6 ± 22.3%，29.5 ± 19.4%，1.8 ± 3.6%，1.0 ± 1.1%，2.1 ± 0.7% respectively, for HSC1, 2, HPC1, 2, 3, 4. Day 12 CFU-S assay showed that the number of colonies were 1.6±1.1，3.2±0.5，2.7±2.0，5±0.0，1.6±1.5，0.5±0.5, respectively, for HSC1, 2, HPC1, 2, 3, 4. On the day 30 after transplantation with 100 HSC1, 2, HPC1, 2, 3, 4 cells, the percentage of chimerism in PB were 55.0±0.7%，10.0±11.8%，20.6±35.6%, respectively, for HSC1, HSC2 and HPC2. After four months of observation, HSC2 tended to differentiate into lymphoid cells, and the ability of HPC2 to reconstitute blood rapidly declined to disappear. The reconstitution ability of HSC1 has always been at a stable level. Conclusion: This study suggests that LT-HSCs contribute early reconstitution as well as short-term and long-term reconstitution.

Keywords: hematopoietic stem/progenitor cells, early reconstitution, chimerism

D3

Gdf-1 as a Regulator of Sphingolipid Metabolism and Stem Cells in Acute Myeloid Leukemia

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Mutations to epigenetic and spliceosome regulators frequently occur in acute myeloid leukemia (AML) and contribute to the underlying pathobiology of the disease. In addition, recent studies have sought to improve the anti-AML efficacy of sphingolipid-based therapeutics by identifying the underlying mechanisms responsible for dysfunctional sphingolipid metabolism. The present study focused on the bicistronic *Cers1-Gdf1* gene and one of its products, Gdf-1, as a regulator of sphingolipid metabolism and stem cells. Gdf-1 is a member of the TGFβ superfamily of proteins, which has been associated with stem cell regulation. This study utilized mouse models of myelodysplastic syndrome and AML to evaluate regulation of *Cers1-Gdf1* gene expression. These include transgenic $FLT3^{ITD}$ mice, *Asxl1* and *Tet2* deletion mice, as well as *Srsf2* mutant mice. Altered expression of sphingolipid metabolic genes was observed in hematopoietic cells isolated from transgenic mice with deletions in epigenetic regulators or spliceosome mutation, as well as $FLT3^{ITD}$ AML mice treated with or without the hypomethylating agent decitabine. Notably, the expression of *Gdf1* was inversely related to the expression of *Ugcg* (glucosylceramide synthase) and *Sgms1* (sphingomyelin synthase 1), both members of the ceramide neutralization pathway. We further observed that treatment with recombinant Gdf-1 downregulated *Ugcg* and *Sgms1* expression and reduced the prevalence of quiescent hematopoietic progenitors while promoting differentiation. Lastly, recombinant Gdf-1 treatment was observed to enhance the *in vivo* efficacy of the standard-of-care AML therapeutic cytarabine. Overall, this study provided evidence that regulation of *Gdf1* can be linked to ceramide neutralization and stem cell pathways in AML. Therefore, dysfunctional *Gdf1* expression may represent a pathway governing the development of stem cell malignancies like AML as well as to enhanced ceramide metabolism that otherwise limits therapeutic efficacy. Moreover, recombinant Gdf-1 may be developed as a combinatorial ceramide-regulating and stem cell-regulating therapeutic for the treatment of hematological malignancies. This work was supported by 7K22-CA190674-02 from the National Cancer Institute.

Keywords: Gdf-1, hematopoietic stem cell, acute myeloid leukemia

D4

Maintenance of human haematopoietic stem and progenitor cells in vitro using a chemical cocktail

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Content: Identification of effective culture conditions to maintain and possibly expand human HSPCs *in vitro* is an important goal. Recent advances highlight the efficacy of chemicals in maintaining and converting cell fates. Methods: We screened 186 chemicals and found a combination of CHIR-99021, Forskolin and OAC1(CFO) maintained human CD34-positive cells *in vitro*. Efficiency of the culture system was characterized using flow cytometry for CD34-positive cells, a colony-forming assay, xeno-transplants and single cell RNA-seq analysis. Results: Human CD34-positive cells treated with this combination CFO had enhanced expression of human HSPCs markers and increased haematopoietic re-populating ability in immune deficient mice. Single cell RNA-seq analyses showed the *in vitro* cultured human CD34-positive cells were heterogeneous. CFO supports human CD34-positive cells maintenance by activating *HOXA9*, *GATA2* and AKT-cAMP signaling pathway. Conclusion: We found human HSPCs are maintained *in vitro* in cultures with CFO. Using chemicals to maintain or increase HSPCs offers a new approach to solve problems in haematopoietic cell transplants and gene therapy.

Keywords: human hematopoietic stem cell, expansion, chemical cocktail

D5

The molecular mechanisms of irradiation induced hematopoietic stem cell injury

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Content: Long-term myelosuppression is the main manifestation of long-term bone marrow injury induced by irradiation (IR). Our previous studies had found that IR could cause hematopoietic stem cell (HSC) senescence, which was mainly manifested by reduced self-renewal capacity, shift of differentiation potential, and homeostasis maintenance. In order to investigate the molecular mechanisms of HSC injury induced by IR, we screened differential genes through the data of gene chip and expression database, and sorted cells for verification, which could provide new clues for preventing long-term myelosuppression induced by IR and studying the aging of hematopoietic system. Methods: C57BL/6 mice were exposed to 6Gy γ-rays of total body irradiation (TBI) to establish hematopoietic radiation injury models, and euthanized at 4 weeks, 8 weeks, 16 weeks and 24 weeks after TBI for analysis. Meanwhile, 6-8 weeks C57BL/ 6 mice and 24 months C57BL/6 mice were respectively used as young and old mouse models. The phenotype of bone marrow cells was analyzed as long-term HSC (LT-HSC), short-term HSC (ST-HSC), common myeloid progenitor (CMP), common lymphoid progenitor (CLP), granulocyte-macrophage progenitor (GMP), and megakaryocyte-erythroid progenitor (MEP) and hematopoietic progenitor cell (HPC). The expression levels of candidate genes in sorted lineage- sca-1+ c-kit+ (LSK) were detected by RT-PCR. Results: (1) Based on the analysis of gene expression database, 34 candidate genes related to HSC injury induced by IR were screened, such as Osgin1, Atf7, Ms4a3, Stab1 and Gsdmd. (2) Compared with the control group, the ratio of LSK cells of IR group had no alternation at different time points post IR exposure; the ratio of HPC of IR group was significantly decreased at 4 weeks and returned to normal levels later; the ratio of CLP significantly decreased ,while there were no changes in ratio of LT-HSC, CMP, GMP, and MEP at 16 weeks and 24 weeks from irradiated mice post IR. (3) The expression levels of Ms4a3 were significantly increased and the expression levels of Stab1 was decreased, while the expression levels of Gsdmd had no changes in sorted LSK cells from irradiated mice at 8 weeks, 16 weeks and 24 weeks post IR. (4) Compared with young mice, the expression levels of Ms4a3 in sorted LT-HSC, ST-HSC and HPC of aged mice were significantly increased, and the expression levels of Stab1 and Gsdmd in sorted LT-HSC were significantly decreased but didn't change both in sorted ST-HSC and HPC of aged mice. Conclusion: The expression changes of Ms43a, Stab1 and Gsdmd in irradiation induced HSC injury might be

involved in lineage bias of HSC differentiation, which were consistent with the changes in aged mice. The regulation of these genes in irradiation induced HSC injury remained to be further studied.

Keywords: hematopoietic stem cell, long-term myelosuppression, differentiation shift

D6

Hematopoietic stem cells exist in Mac-1- or CD41-negative and low fractions in adult mouse bone marrow

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Content: Hematopoietic stem cells (HSCs) have the self-renewal potential with differentiation potential into all blood cell types. HSCs are extremely rare in bone marrow, like one in 10^5 cells. Purification of HSCs depends on the expression of combinations of surface markers on HSCs to distinguish them from other cell types. Whether Mac-1 and CD41 are expressed on HSCs is controversial. This study addressed this issue, and asked whether these markers can be used for the further purification of HSCs. Methods: Cells were purified from bone marrow cells of Ly-5.1 C57BL/6 mice and used as donor cells. A small number of purified cells or single cells with $5x10^5$ competent cells were injected into lethally irradiated Ly-5.2 C57BL/6 mice. Peripheral blood was obtained 1, 3, 6, and 9 months post-transplantation to analyze engraftment of donor derived cells. 3-lineage (L_3) cocktail consisted of B220, Gr1 and Ter119 antibodies. 7-lineage (L_7) cocktail consisted of Mac1, CD3, CD4, CD8, B220, Gr-1, and Ter119 antibodies. Results: We first compared the depletion of 3 and 7 lineages from $CD150^+CD41^-CD34^-$SK cells by transplantation assay. Results showed the similar levels of long-term reconstitution between 3 and 7 lineage-depletion. We analyzed the expression of Mac1, CD3, CD4, CD8, B220, Gr-1, or Ter119 in $CD201^+CD150^+CD41^-CD34^-L_3$SK cells. A significant portion of these cells expressed a low level of Mac-1 but not the others. We compared Mac-negative or Mac-low cells by transplantation. Results showed the similar levels of long-term reconstitution between Mac-1-negative and low cells. We analyzed the expression of CD41 in $CD150^+CD34^-L_3$SK cells. A significant portion of these cells expressed CD41. We separated these cells into CD41-negative, low, and high fractions, and performed transplantation. Results showed the similar levels of long-term reconstitution between CD41-negartive and low cells, but no reconstitution with CD41-high cells. Conclusion: In conclusion, this study showed that depletion of $Mac1^{low}$ and $CD41^{low}$cells in HSC purification inadvertently discards a significant population of HSCs. Despite of a long history of lineage depletion using 6 or 7 lineages, we recommend to use 3 lineages. However, whether Mac-1 and CD41 are used does not significantly change the purity of HSCs.

Keywords: Mac1, CD41, Hematopoietic stem cells

D7

The mechanism of A-to-I RNA editing of ADAR1 in hematopoiesis

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Content: In the process of adult hematopoiesis, the differentiation of hematopoietic stem and progenitor cells (HSPCs) is accurately regulated. Besides the transcription level, post transcriptional regulation also plays an indispensable role in hematopoiesis. Adenosine Deaminase Acting on RNA 1 (ADAR1) converts adenosine (A) to inosine (I) on double-stranded RNAs, which affects RNA stability or changes the amino acid sequences, and so on. ADAR1-deficient HSPCs were incapable of reconstituting irradiated recipients and forming differentiated colonies. However, the mechanism under ADAR1 regulating the differentiation of HSPCs is still unclear. In this study, we try to unearth the mechanism of ADAR1 in hematopoiesis based on RNA sequencing of hematopoietic lineages. Methods: Strand-specific, ribo-minus RNA-Seq libraries were prepared from 12 stages of hematopoietic cells derived from flow cytometry. Genomic DNA was obtained from HSPCs for the library preparation of genome sequencing. A-to-I editing sites were called by in-house scripts as previously reported and verified by sanger sequencing. To identify editing sites that are specifically edited in only one stage/group, we applied the ROKU R package to rank sites by overall cell specificity using Shannon entropy and detected cell type/group specificity. If any exists, using an outlier detection method. Then we used Snpeff to determine which editing sites could resulted in missense mutation in coding region. Then, a missense A-to-I group specific editing sites were selected. The site of Gene A was hyperedited in HSPCs with editing sites located in CDS regions. We knocked down the expression of gene A in HSPCs to test its function in hematopoiesis. We also wanted to see if the editing of gene A contributed to its function, so we overexpress the edited gene A as well as the wild-type gene A in HSPCs. In 3'UTR regions, we discovered the expression of several genes varied inversely with their editing level. We used q-PCR and western bloting to authenticate it. Results: There were 30796 editing sites across 12 stages of hematopoietic cells. 54.9% editing sites were located in intron while only 1.51% of them located in coding region. The overall editing level of intron was the highest while the editing level in coding region was the lowest. 74.41% editing sites were located in Alu region, in contrast, 28.59% editing sites were located in non Alu region. Moreover, the Alu editing sites located in intron were significantly enriched while non Alu editing sites in coding region were significantly enriched. The landscape of 12 stages hematopoietic cells editome displays stage specific RNA editing. Moreover, there were group specific RNA editing.

Both stage specific RNA editing and group specific RNA editing were associated with hematopoiesis. In coding region, both stage specific RNA editing and group specific RNA editing resulted in missense mutation. Group specific RNA editing of gene A, which is hyperedited in HSPCs, could cause a conformational change and induce a cytoplasmic to nuclear translocation. RNAi of gene A in HSPCs could impair the function of HSPCs, including reconstituting irradiated recipients as well as forming differentiated colonies, the same with ADAR1-deficient HSPCs. Overexpression of edited gene A but not wild-type gene A, could enhance the function of HSPCs. In 3'UTR regions, Several genes, whose expressions varied inversely with their editing frequencies, may prevent the inappropriate activation of the immune pathway through editing of their repetitive elements. Conclusion: The requirement for ADAR1 within the HSPCs has not been explored in detail. We sorted high purity of 12 lineages of hematopoietic cells by flow cytometry to prepare ribo-minus, strand specific RNA-seq libraries. We called editing sites by in-house scripts and discovered numerous editing sites. Then we pictured the landscape of RNA editome and found both stage specific RNA editing sites and group specific RNA editing sites were associated with hematopoiesis. Meanwhile, we found stage specific and group specific RNA editing sites could cause missense mutation in coding regions. ADAR1-deficient HSPCs were incapable of reconstituting irradiated recipients and forming differentiated colonies. We hypothesized that some genes, which had important functions to HSPCs, were edited by ADAR1. Gene A was hyperedited in HSPCs, and RNAi of gene A in HSPCs could impair the function of HSPCs, the same with ADAR1-deficient HSPCs. Compared to overexpression of wild-type gene A, overexpression of edited gene A could enforce the function of HSPCs, such as reconstituting irradiated recipients, and so on. In 3'UTR regions, editing of the repetitive elementsmay prevent the inappropriate activation of the immune pathway to provide a suitable microenvironment for hematopoiesis.

Keywords: ADAR1, hematopoiesis, RNA editing

D8

Bone marrow endothelial cell-derived interleukin-4 exacerbated thrombocytopenia in acute myeloid leukemia

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Content: Thrombocytopenia is a major and fatal complication in the patients with acute leukemia. However, the mechanisms underlying defective thrombopoiesis in leukemia have not been fully elucidated. Methods: In this study, we employed the non-irradiated MLL-AF9 induced acute myeloid leukemia (AML) murine model as previously reported (Cheng et al, 2015) to define how megakaryopoiesis is suppressed during AML development. Results: The AML mice exhibited a progressive decrease of platelet count during disease development (1315±80.65 vs 143.3±68.01×10^3 /μl, P<0.001). We also measured the frequency of hematopoietic stem and progenitor (HSPC) subsets in the residual hematopoietic cells along the path of megakaryopoiesis and found that the frequency of megakaryocytic/erythroid progenitors (preMegEs) declined dramatically (14±4% of healthy state), whereas the frequency of Lin-Sca-1+c-kit+CD150+ hematopoietic stem cells (HSCs) gradually increased (3.1±0.4 folds of healthy control), suggesting a blockade of megakaryopoiesis from CD150+ HSCs. Consistently, we observed reduced megakaryocyte colony forming ability (72.3±3.4 vs 89.0±1.0%, P=0.097) *in vitro* and defective platelet reconstitution potential after transplantation of CD150+ LKS cells (4.3±0.8 vs 13.8±2.3%, P=0.002) from AML mice. To search for a novel soluble factor involved in the suppressive megakaryopoiesis, we detected elevated Interleukin 4 (IL4) protein level in bone marrow plasma and upregulated IL4 mRNA expression in bone marrow endothelial cells in the AML model. Notably, platelet counts(325±58 vs 991±87 X10^{12}/L, P<0.001)and the frequency of megakaryocytes(0.008±0.001 vs 0.02±0.002%, P<0.001)in the mice receiving IL4 dropped dramatically. The frequency of preMegEs(0.089±0.003 vs 0.19±0.026%, P=0.0043)decreased. These observations indicated that IL4 exerted direct inhibitory effects on megakaryocytic differentiation of HSPCs and megakaryocyte maturation, and IL4 treatment could phenocopy defective thrombopoiesis in AML to some extent. Moreover, administration of IL4 neutralizing antibody partially recovered the yield of megakaryocyte colonies in the presence of leukemia bone marrow plasma(32.5±1.555 vs 46.5±2.598, P=0.0036). Conclusion: In summary, our current study demonstrates that defective megakaryopoiesis in AML was at least partially contributed by the suppression of HSC differentiation toward megakaryocytic lineage and also reveals a previously unrecognized link between IL4 signaling and megakaryopoiesis.

Keywords: AML, thrombocytopenia, IL4

D9

Sequential cellular niches control the generation of enucleated erythrocytes from human pluripotent stem cells

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Human pluripotent stem cells (hPSCs) provide a powerful platform for *in vitro* generation of red blood cells to overcome blood supply shortages. Although previous studies have demonstrated that hPSCs have the ability to differentiate into erythroid cells, the generation of enucleated erythrocytes has still been challenging. Here, we demonstrated that providing two sequential cellular niches at different developmental stages promoted erythroid differentiation and erythrocyte maturation to generate β-globin expressing enucleated erythrocytes. Whereas endothelial cells (ECs) provided extrinsic signals to promote endothelial-to-hematopoietic transition of hemogenic endothelial (HE) cells to generate definitive hematopoietic progenitor cells, a further differentiation of EC-primed erythroid progenitors on OP9 bone marrow stromal cells generated approximately 75% enucleated erythrocytes with ~40% HbA(α2β2) expression. Omitting either of the cellular niches or switching the order of co-culture with ECs and OP9 cells significantly reduced the efficiency of mature erythrocyte generation. Furthermore,blockage of Notch signaling during erythroid differentiation from HE cells significantly reduced EC-niche potential for the generation of definitive erythroid progenitors that are capable of enucleation, whereas ectopic expression of Notch ligand, DLL1, in OP9 cells impaired OP9-niche ability to promote erythroid maturation, including adult-type hemoglobin expression and enucleation. Our studies demonstrated for the first time that cellular niches played crucial and distinct roles in the different stages of erythroid development, and that an unambiguously constructed sequential cellular niches *in vitro* enabled generation of enucleated erythrocytes from hPSCs. Moreover, we found that a balanced positive and negative niche-dependent Notch signaling was required for definitive erythroid fate and erythroid maturation. Collectively, our sequential multi-niche model not only presents a platform for further investigation of erythroid development and erythroid disorders but also provides a new strategy for bypassing a default primitive hematopoietic pathway to acquire definitive hematopoietic stem cells from hPSCs.

Keywords: Hematopoiesis, Stem Cells, Erythrocyte Enucleation

D10

MEIS2 Regulates Endothelial to Hematopoietic Transition of Human Embryonic Stem Cells by Targeting TAL1

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Despite considerable progress in the development of methods for hematopoietic differentiation, efficient generation of transplantable hematopoietic stem cells (HSCs) and other genuine functional blood cells from human embryonic stem cells (hESCs) is still unsuccessful. Therefore, a better understanding of the molecular mechanism underlying hematopoietic differentiation of hESCs is highly demanded. In this study, by using whole-genome gene profiling we identified Myeloid Ectopic Viral Integration Site 2 homolog (MEIS2) as a potential regulator of hESC early hematopoietic differentiation. We deleted MEIS2 gene in hESCs using the CRISPR/CAS9 technology and found that MEIS2 deletion impairs early hematopoietic differentiation from hESCs. Furthermore, MEIS2 deletion suppresses hemogenic endothelial specification and endothelial to hematopoietic transition (EHT), leading to the impairment of hematopoietic differentiation. Mechanistically, TAL1 acts as a downstream gene mediating the function of MEIS2 during early hematopoiesis. Interestingly, unlike MEIS1, MEIS2 deletion exerts minimal effects on megakaryocytic differentiation and platelet generation from hESCs. Together, our findings advance the understanding of human hematopoietic development and may provide new insights for large-scale generation of functional blood cells for clinical applications.

Keywords: MEIS2, EHT, hematopoiesis

D11

Long Non-Coding RNA-Dependent Mechanism to Regulate Heme Biosynthesis and Erythrocyte Development

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In addition to serving as a prosthetic group for enzymes and a hemoglobin structural component, heme is a crucial homeostatic regulator of erythroid cell development and function. While lncRNAs modulate diverse physiological and pathological cellular processes, their involvement in heme-dependent mechanisms is largely unexplored. In this study, we elucidated a lncRNA (UCA1)-mediated mechanism that regulates heme metabolism in human erythroid cells. We discovered that UCA1 expression is dynamically regulated during human erythroid maturation, with a maximal expression in proerythroblasts. UCA1 depletion predominantly impairs heme biosynthesis and arrests erythroid differentiation at the proerythroblast stage. Mechanistic analysis revealed that UCA1 physically interacts with the RNA-binding protein PTBP1, and UCA1 functions as an RNA scaffold to recruit PTBP1 to ALAS2 mRNA, which stabilizes ALAS2 mRNA. These results define a lncRNA-mediated posttranscriptional mechanism that provides a new dimension into how the fundamental heme biosynthetic process is regulated as a determinant of erythrocyte development.

Key words: UCA1, heme metabolism, ALAS2, posttranscriptional regulation, PTBP1, GATA1

D12

MEIS1 Regulates Hemogenic Endothelial Generation, Megakaryopoiesis and Thrombopoiesis in Human Pluripotent Stem Cells by Targeting TAL1 and FLI1

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Human pluripotent stem cells (hPSCs) provide an unlimited source for generating various kinds of functional blood cells. However, efficient strategies for generating large-scale functional blood cells from hPSCs are still lacking, and the mechanism underlying human hematopoiesis remains largely unknown. In this study, we identified Myeloid Ectopic Viral Integration Site 1 homolog (MEIS1) as a crucial regulator of hPSC early hematopoietic differentiation. MEIS1 is vital for specification of $APLNR^+$ mesoderm progenitors to functional hemogenic endothelial progenitors (HEPs), thereby controlling formation of hematopoietic progenitor cells (HPCs). TAL1 mediates the function of MEIS1 in HEP specification. In addition, MEIS1 is vital for megakaryopoiesis and thrombopoiesis from hPSCs. Mechanistically, *FLI1* acts as a downstream gene necessary for the function of MEIS1 during megakaryopoiesis. Thus, MEIS1 controls human hematopoiesis in a stage-specific manner and can be potentially manipulated for large-scale generation of HPCs or platelets from hPSCs for therapeutic applications in regenerative medicine.

Keywords: MEIS1, Hemogenic Endothelial, hPSC

D13

MSX2 Initiates and Accelerates Mesenchymal Stem Cell Specification of hPSCs by Regulating TWIST1 and PRAME

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The gap in knowledge of the molecular mechanisms underlying differentiation of hPSCs into the mesenchymal cell lineages hinders the application of hPSCs for cell-based therapy. In this study, we identified a critical role of muscle segment homeobox 2 (MSX2) in initiating and accelerating the molecular program that leads to mesenchymal stem/stromal cell (MSC) differentiation from hPSCs. Genetic deletion of MSX2 impairs hPSC differentiation into MSCs. When aided with a cocktail of soluble molecules, MSX2 ectopic expression induces hPSCs to form nearly homogenous and fully functional MSCs. Mechanistically, MSX2 induces hPSCs to form neural crest cells, an intermediate cell stage preceding MSCs, and further differentiation by regulating TWIST1 and PRAME. Furthermore, we found that MSX2 is also required for hPSC differentiation into MSCs through mesendoderm and trophoblast. Our findings provide novel mechanistic insights into lineage specification of hPSCs to MSCs and effective strategies for applications of stem cells for regenerative medicine.

Keywords: MSX2, Mesenchymal Stem Cell , hPSC

D14

Thrombopoietin knock-in augments platelet generation from human embryonic stem cells

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Refinement of therapeutic-scale platelet production in vitro will provide a new source for transfusion in patients undergoing chemotherapy or radiotherapy. However, procedures for cost-effective and scalable platelet generation remain to be established. In this study, we established human pluripotent stem cell (hPSCs) lines containing knock-in of Thrombopoietin (TPO) and tested their potency for platelet generation. TPO knock-in exerts minimal effects on pluripotency but enhances early hematopoiesis and generation of more hematopoietic progenitor cells (HPCs). More importantly, upon its knock-in, TPO augments megakaryocytic differentiation and platelet generation. In addition, the platelets derived from hPSCs in vitro are functionally and morphologically comparable to those found in peripheral blood. Furthermore, TPO knock-in can partially replace the large quantities of extrinsic TPO necessary for platelet generation. Our results demonstrate that autonomous production of cytokines in hPSCs via CRISPR-Cas9 genome editing may become a powerful approach for cost -effective and large-scale platelet generation in translational medicine.

Keywords: Thrombopoietin, Knock-in, embryonic stem cells

D15

R-spondin2 and LGR4 facilitate hematopoietic differentiation of hPSCs by independently regulating TGF-beta signaling

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Attempts of generating genuine hematopoietic stem cells (HSCs) and functional blood cells from human pluripotent stem cell (hPSCs) remain largely unsuccessful, mainly due to the lack of understanding of the regulatory network of human hematopoiesis. In this study, we identified leucine-rich repeat-containing G protein-coupled receptors 4 (LGR4) and R-spondin2 as essential regulators of early hematopoietic differentiation of hPSCs. Deletion of LGR4 severely impairs mesoderm development, causing much fewer APLNR^{+}lateral plate mesoderm cells and thereby limiting hematopoietic differentiation of hPSCs both in vitro and in vivo. Supply of R-spondin2 exogenously promotes hPSC hematopoietic differentiation by augmenting mesoderm differentiation. Surprisingly, LGR4 appears to act independently of R-spondin2, because depletion of LGR4 does not abolish the enhancement of hematopoietic differentiation induced by R-spondin2. By concomitantly deleting LGR4 and LGR5, we further illustrate that LGR4, but not LGR5, modulates human pluripotent stem cell hematopoietic differentiation. At the mechanistic level, R-spondin2 and LGR4 regulate both Wnt and TGFβ signaling to control hematopoietic differentiation. Together, we reveal vital roles of R-spondin2 and LGR4 in hematopoietic development, which may prove to be invaluable for large-scale generation of functional hematopoietic progenitors for translational medicine.

Keywords: hPSC, hematopoietic differentiation, LGR4

D16

Genetic rescue of lineage-balanced blood cell production reveals a crucial role for STAT3 anti-inflammatory activity in hematopoiesis

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Blood cell formation must be appropriately maintained throughout life to provide robust immune function, hemostasis, and oxygen delivery to tissues, and to prevent disorders that result from over or underproduction of critical lineages. Persistent inflammation deregulates hematopoiesis by damaging hematopoietic stem and progenitor cells (HSPCs), leading to elevated myeloid cell output and eventual bone marrow failure. Nonetheless, antiinflammatory mechanisms that protect the hematopoietic system are understudied. The transcriptional regulator STAT3 has myriad roles in HSPC-derived populations and non-hematopoietic tissues, including a potent anti-inflammatory function in differentiated myeloid cells. STAT3 anti-inflammatory activity is facilitated by STAT3-mediated transcriptional repression of Ube2n, which encodestheE2ubiquitin-conjugatingenzymeUbc13involvedinproinflammatory signaling. Here we demonstrate a crucial role for STAT3 anti-inflammatory activity in preservation of HSPCs and lineage-balanced hematopoiesis. Conditional Stat3 removal from the hematopoietic system led to depletion of the bone marrow lineage- Sca-1+ c-Kit+ CD150+ CD48- HSPC subset (LSK CD150+ CD48- cells), myeloid-skewed hematopoiesis, and accrual of DNA damage in HSPCs. These responses were accompanied by intrinsic transcriptional alterations in HSPCs, including deregulation of inflammatory, survival and developmental pathways. Concomitant Ube2n/Ubc13 deletion from Stat3-deficient hematopoietic cells enabled lineage-balanced hematopoiesis, mitigated depletion of bone marrow LSK CD150+ CD48- cells, alleviated HSPC DNA damage, and corrected a majority of aberrant transcriptional responses. These results indicate an intrinsic protective role for STAT3 in the hematopoietic system, and suggest this is mediated by STAT3-dependent restraint of excessive pro-inflammatory signaling via Ubc13 modulation.

Keywords: HSPC, inflammation

D17

Rheb1 loss leads to increased hematopoietic stem cell proliferation and myeloid-biased differentiation in vivo

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Hematopoietic stem cells constitute a unique subpopulation of blood cells that have the ability to give rise to all types of mature cells in response to physiological demands. However, the intrinsic molecular machinery that regulates this transformative property remains elusive. In this paper, we demonstrate that small GTPase Rheb1 is a critical regulator of proliferation and differentiation of hematopoietic stem cells *in vivo*. Rheb1 deletion led to increased phenotypic hematopoietic stem cells/hematopoietic progenitor cells proliferation under the steady state condition. Over-proliferating Rheb1-deficient hematopoietic stem cells were severely impaired in functional repopulation assays, and they failed to regenerate blood system when challenged with hematopoietic ablation by sublethal irradiation. Additionally, it was discovered that Rheb1 loss caused neutrophil immaturation by reducing mTORC1 activity, and that activation of the mTORC1 signaling pathway by mTOR activator 3BDO partially restored the maturation of Rheb1-deficient neutrophils. Rheb1 deficiency led to a progressive enlargement of hematopoietic stem cells population and eventual excessive myeloproliferation *in vivo*, including an overproduction of peripheral neutrophils and an excessive expansion of extramedullary hematopoiesis. Moreover, the low expression of RHEB was correlated with poor survival of acute myeloid leukemia patients with normal karyotype. Our results thus demonstrate a critical and unique role for Rheb1 in maintaining proper hematopoiesis and myeloid differentiation.

Keywords: Rheb1, mTORC1, HSC

D18

Mettl3–Mettl14 methyltransferase complex regulates the quiescence of adult hematopoietic stem cells

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N6-methyladenosine (m^6A) is a common modification of mRNA that is catalyzed by the Mettl3–Mettl14 methyltransferase complex, with WTAP as its regulatory subunit. The physiological importance of m^6A has been evidenced by its pivotal roles in tissue development and differentiation. However, it is poorly understood how m^6A regulates the self-renewal of tissue-specific stem cells. In this study, using mouse hematopoietic system as a model, we characterized the individual and combined functions of Mettl3 and Mettl14 in regulating hematopoietic stem cell (HSC) self-renewal in adult bone marrow. We found that conditional deletion of *Mettl3* from hematopoietic cells in adult bone marrow led to a ~10-fold expansion of the $CD48^-CD150^+Sca\text{-}1^+c\text{-}kit^+Lineage^-$ phenotypic HSCs, but a severe depletion of long-term reconstituting HSCs. Mice with *Mettl14* knockout showed a milder defect in the reconstituting capacity of bone marrow HSCs. Mice with *Mettl3* and *Mettl14* compound knockouts phenocopied those with *Mettl3* single knockout in terms of their HSC frequency. m^6A inhibited HSC cycling and promoted the expression of genes that regulate HSC quiescence. Thus, our work revealed that the Mettl3-Mettl14 methyltransferase complex is essential for the self-renewal of adult HSCs. We also provided evidence that Mettl3 dominates the functions of m^6A methyltransferase complex in HSC regulation.

Keywords: hematopoietic stem cells, N6-methyladenosine, quiescence

D19

Luteinizing hormone signaling restricts hematopoietic stem cell expansion during puberty

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The number and self-renewal capacity of hematopoietic stem cells (HSCs) are tightly regulated at different developmental stages. Many pathways have been implicated in regulating HSC development in cell autonomous manners; however, it remains unclear how HSCs sense and integrate developmental cues. In this study, we identified an extrinsic mechanism by which HSC number and functions are regulated during mouse puberty. We found that the HSC number in postnatal bone marrow reached homeostasis at 4 weeks after birth. Luteinizing hormone, but not downstream sex hormones, was involved in regulating HSC homeostasis during this period. Expression of luteinizing hormone receptor (Lhcgr) is highly restricted in HSCs and multipotent progenitor cells in the hematopoietic hierarchy. When Lhcgr was deleted, HSCs continued to expand even after 4 weeks after birth, leading to abnormally elevated hematopoiesis and leukocytosis. In a murine acutemyeloid leukemia model, leukemia development was significantly accelerated upon Lhcgr deletion. Together, our work reveals an extrinsic counting mechanism that restricts HSC expansion during development and is physiologically important for maintainingnormal hematopoiesis and inhibiting leukemogenesis.

Keywords: hematopoietic stem cell, luteinizing hormone, puberty

D20

PPM1K regulates hematopoiesis and leukemogenesis through CDC20-mediated ubiquitination of MEIS1 and p21

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In addition to acting as building blocks for biosynthesis, amino acids might serve as signaling regulators in various physiological and pathological processes. However, it remains unknown whether amino acid levels affect the activities of hematopoietic stem cells (HSCs). By using a genetically encoded fluorescent sensor of the intracellular levels of branched-chain amino acids (BCAAs), we could monitor the dynamics of BCAA metabolism in HSCs. A mitochondrial-targeted 2C-type Ser/Thr protein phosphatase (PPM1K) promotes the catabolism of BCAAs to maintain MEIS1 and *P*21 levels by decreasing the ubiquitination-mediated degradation controlled by the E3 ubiquitin ligase CDC20. PPM1K deficiency led to a notable decrease in MEIS1/*P*21 signaling to reduce the glycolysis and quiescence of HSCs, followed by a severe impairment in repopulation activities. Moreover, the deletion of Ppm1k dramatically extended survival in a murine leukemia model. These findings will enhance the current understanding of nutrient signaling in metabolisms and activities of stem cells.

Keywords: branched-chain amino acids, PPM1K, ubiquitination

D21

PTIP Is Required for Maintenance of Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) is a common fatal hematopoietic malignancy, characterized by the accumulation of clonal myeloid progenitor cells showing inability to differentiate into mature blood cells. Although reductions in leukemia blasts can be achieved initially with current chemotherapy in most patients, long-term outcomes have not improved significantly for the last two decades. In addition, increasing evidence indicate that the existence of leukemia stem cell (LSCs) is the major reason for AML relapse in clinic, due to their insensitivity to drug treatment. Thus, development of novel therapeutic strategy targeting AML LSCs is highly urgent, which relies on fully understanding the molecular pathogenesis of AML and on exploring the molecular mechanisms of LSCs maintenance. In here, we found that PTIP (also called PAXIP1, Pax transactivation domain-interacting protein) affects LSC self-renewal and is required for AML survival. First, we observed higher level of *PITP* transcript in AML patient-derived leukemia cells compared to normal controls, which is consistent with the finding showing elevated *PTIP* expression in AML cells by analyzing public database. We further determine the role of PTIP in human and murine myeloid leukemia cells using shRNAs to ablate its expression. Compared with the scramble control, all shPTIP markedly deleted *PTIP* expression, *PTIP* knockdown significantly blocked cell growth, inhibited clonogenic ability, accompanying with increased apoptosis. We observed a consistent effect by CRISPR-Cas9 mediated gene editing. These results suggest that PTIP plays an important role in AML. Previous studies showed that PTIP is not only a critical component of MLL3/4 COMPASS complex, but also involves in DNA damage repair through interacting with 53BP1. We found that loss of PTIP caused accumulation of DNA damage in AML cells showing increased DNA damage response signaling, such as pDNA-PK, *P-P*53, pH2AX. In addition, PTIP deletion significantly suppressed H3K4me3 level but did not affect H3K4me2 and H3K4me1 in leukemia cells. These results indicate that PTIP affects H3K4 methylation in leukemia cells. Together, our results may provide first evidence for a functional role of PTIP in AML maintenance by epigenetic alteration and affecting genome stability of AML cells.

Keywords: Acute myeloid leukemia, eukemia stem cell, PTIP

D22

TET2 Regulates Osteoclast Differentiation by Interacting with RUNX1 and Maintaining Genomic 5-Hydroxymethylcytosine

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As a dioxygenase, Ten-eleven translocation 2 (TET2) protein catalyzes subsequent steps of 5-Methylcytosine (5-mC) oxidation. Loss-of-function mutations in *TET2* are frequent in both myeloid and lymphoid malignancies. *TET2* mutations also occur in healthy elderly individuals with clonal hematopoiesis. Both *Tet2*$^{-/-}$ mice and patients with *TET2* mutations often have increased numbers of monocyte/macrophage osteoclast precursors, due to a skewed differentiation of HSC/HPCs to monocytic lineage. The role of TET2 in the self-renewal, proliferation and differentiation of hematopoietic stem cells was extensively investigated and well documented, but its impact on mature hematopoietic cells is not well-characterized. Here we show that TET2 plays an essential role in osteoclastogenesis. Despite an increased population of precursors, the number of osteoclasts is significantly decreased in *Tet2*$^{-/-}$ mice, likely due to the impairment of osteoclast differentiation. We found that *Tet2*$^{-/-}$ and *Tet2*$^{f/f}$*;LysMCre* mice showed mild osetopetrosis, accompanied by decreased osteoclast numbers *in vivo*. Using *in vitro* osteoclast differentiation assay, we found that deletion of *Tet2* impairs osteoclast precursor cell (macrophage) differentiation and maturation into bone-resorbing osteoclasts. As revealed by the RNA-seq analysis, *Tet2* loss in osteoclast precursor cells altered the expression of a set of genes implicated in osteoclast differentiation, such as *Cebpa* and *Nfkbiz* (through inhibition of NFκB activity). The expression level of these genes were confirmed to be significantly different by quantitative-PCR. It has been shown that *Cebpa*$^{-/-}$ mice develop a severe osteopetrotic phenotype because of defective osteoclastogenesis. NFκB is one of the most important transcription factors for osteoclast differentiation activated by RANKL. Inactivation of NFκB pathway by interruption of either IKKα or KKβ resulted in the disruption of osteoclast differentiation. Therefore, the down-regulation of *Cebpa* and up-regulation of *Nfkbiz* in *Tet2*$^{-/-}$ osteoclast precursor cells may both contribute to the impaired osteoclast differentiation and the osteopetrotic bone phenotype in *Tet2* deficient mice. Genome-wide 5-hydroxymethylcytosine (5hmC) profiling (hMe-Seal) and high-throughput sequencing further revealed that *Tet2* deletion also led to a genome-wide alteration of 5hmC in osteoclast precursor cells with enrichment of

differential hydroxymethylated regions in specific sets of genes implicated in osteoclast differentiation. However, there was no overall linear correlation between gene expression changes and 5hmC alteration in *Tet2*$^{-/-}$ vs WT osteoclast precursor cells. This is consistent with previous findings that no linear correlation between gene expression changes and 5hmC alteration in mouse LK cells and ESCs. Thus promotes us to explore the potential interaction protein of TET2. To survey potential TET2 binding partners, we performed anti-FLAG-mediated affinity purifications using MEL cells stably expressing FLAG-TET2 (~4 fold of endogenous TET2 expression). LC-MS/MS analysis identified a list of high-confidence candidate TET2-interacting proteins, including known TET2-interactors such as OGT and several unknown candidates, including RUNX1. The interaction of TET2 with RUNX1 was confirmed by reciprocal co-immunoprecipitation using HEK293T cells overexpressing FLAG-TET2. Furthermore, RUNX1 binding motifs are relatively enriched in the FLAG-TET2 ChIP-seq peak regions, suggesting that TET2 may regulate the transcriptional activity of RUNX1. Indeed, luciferase reporter assays showed that TET2 negatively modulates RUNX1 transcriptional activity. It has been shown that the deletion of *Runx1* in hematopoiesis in adult mice produces a myeloproliferative phenotype and that *Runx1* loss accelerates osteoclastogenesis. These data suggests that *Tet2* loss activates RUNX1 transcriptional activity, contributing to the impaired osteoclast differentiation. In conclusion, our studies demonstrate a novel molecular mechanism controlling osteoclast differentiation and function by TET2, through interactions with RUNX1 and the maintenance of genomic 5-hydroxymethylcytosine. Targeting TET2 and its pathway could be a novel therapeutic strategy for the prevention and treatment of abnormal bone mass caused by osteoclast activity deregulation.

Keywords: Tet2, 5hmC, osteoclast

D23

The effect of NLRP1 in bone marrow environment on reconstruction of bone marrow hematopoietic function following allogeneic hematopoietic stem cell transplantation

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Objective The purpose of this study was to explore the function of NLRP1 in bone marrow environment on recovering of hematopoietic function following allogeneic hematopoietic stem cell transplantation (HSCT)Methods Normal and NLRP1$^{-/-}$ C57BL/6 mice aged from 8 to 10 weeks were transplanted with bone marrow cells from BABL/c mice (named as normal and NLRP1$^{-/-}$ HSCT group respectively). On 7th, 14th, 21st, 28th day after transplantation, peripheral blood cells count, pathology changes in bone morrow, HSC %, neutrophils, macrophages, apoptosis of bone marrow cells and cytokines were measured by HE staining, flow cytometry and ELISA. CFSE-labeling bone marrow cells were transplanted into C57BL/6mice and NLRP1$^{-/-}$mice. On day 3 and 5, CFSE^{+} cells and SDF-1, S1*P* and C1*P* in bone marrow were tested to evaluate cell homing.Results The results showed that bone marrows damage in NLRP1$^{-/-}$ HSCT group was lighter than that in normal HSCT group. The percentages of neutrophils and macrophage in NLRP1$^{-/-}$ HSCT group were lower on day 21. The apoptosis cells % in bone marrows were less in NLRP1$^{-/-}$ HSCT group on day 7, 14 and 21. The contents of IL-1β on the 7, 14, 21 and 28, the contents of IL-18 and IL-6 on the 14, 21 and 28, the contents of TNF-α on the 21 and 28 in the NLRP1$^{-/-}$ HSCT group were lower. Besides, WBC in peripheral blood and HSPC% were also lower in NLRP1$^{-/-}$ HSCT group on the day 7 and 14.Moreover, it showed that CFSE^{+} cells and SDF-1, S1*P* and C1*P* in NLRP1$^{-/-}$ HSCT group were less than those in normal HSCT group.Conclusion Knocking out NLRP1 gene can reduce the inflammatory damage in bone marrow following Allo-HSCT, but it also can delay the recovery of hematopoietic function by reducing the number of homing HSCs following Allo-HSC, which shows that NLRP1 may play an important role in the processes of inflammatory injury and reconstruction bone marrow hematopoietic function.

Keywords: Hematopoietic stem cell transplantation, NLRP1, Homing

D24

High-Level Precise Knockin of iPSCs by Simultaneous Reprogramming and Genome Editing of Human Peripheral Blood Mononuclear Cells

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We have developed an improved episomal vector system for efficient generation of integration-free induced pluripotent stem cells (iPSCs)from peripheral blood mononuclear cells. More recently, we reported that the use of an optimized CRISPR-Cas9 system together with adouble-cut donor increases homology-directed repair-mediated precise gene knockin efficiency by 5- to 10-fold. Here, we report the integrationof blood cell reprogramming and genome editing in a single step.We found that expression of Cas9 and KLF4 using a single vectorsignificantly increases genome editing efficiency, and addition of SV40LT further enhances knockin efficiency. After these optimizations,genome editing efficiency of up to 40% in the bulk iPSC population can be achieved without any selection. Most of the edited cells showcharacteristics of iPSCs and genome integrity. Our improved approach, which integrates reprogramming and genome editing, shouldexpedite both basic research and clinical applications of precision and regenerative medicine.

Keywords: peripheral blood mononuclear cells, CRISPR-Cas9, induced pluripotent stem cells

D25

CD43 expression faithfully marks definitive hematopoietic stem progenitor cells throughout mouse embryonic development

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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. Here, we reported that primitive erythroid progenitors lacked CD43 expression, but definitive hematopoietic progenitor cells resided within $CD43^+$ population. CD43 expression marked the first cohort of HSCs in E10.5 aorta gonad mesonephros (AGM) region. Further, emerging HSCs were enriched within $CD34^+$c-Kit^+ population in head, yolk sac, and placenta.In fetal liver, expanding HSCs also expressed high level CD43.Significantly, we re-defined endothelial cells as $CD31^+CD41^-CD43^-CD45^-Ter119^-$ population in E10-E11 AGM, 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.

Keywords: CD43, definitive hematopoiesis, hematopoietic stem cells

D26

Effects of ectopic expression of miR-99a on human hematopoietic stem/progenitor cells maintenance and differentiation

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Objective: Emerging evidence suggests that microRNAs (miRNAs)have been involved in various physiological and pathological processes, such as cell proliferation, differentiation, metabolism and cancer progression, including leukemogenesis. We previously identified that upregulation of miR-99a was associated with poor prognosis of acute myelogenous leukemia (AML) that results in cell expansion and progression of myeloid leukemia. In this study, by using lentiviral vectors carrying hsa-miR-99a-5*P* (miR-99a) or a scrambled sequence (Ctrl),we aim to clarify the effects of ectopic expression of miR-99a on normal hematopoietic stem/progenitor cells (HSPCs). Methods: To dissect the expression level of miR-99a in different hematopoietic lineages, qPCR analysis was performed in HSPCs, T cells, B cells, granulocytes and monocytes sorted from healthy donors.To determine the potential effects of ectopic expression of miR-99a on cellular function of HSPCs, miR-99a was ectopically expressed in cord blood $CD34^+$cells via stable lentiviral transduction. The characteristics of HSPCs/miR-99a were then examined, including cell proliferation, differentiation, cell cycle, apoptosis, and colony forming assay. Target genes of miR-99a were further determined by RNA-Seq and prediction through different databases and the following qPCR validation.Results: We identified that the expression level of miR-99a was higher in $CD34^+$HSPCs from healthy donors than other mature lineages, indicating that the level of miR-99a were gradually decreased during the differentiation and maturation of normal hematopoietic cells. As mentioned before, the expression of miR-99a was even higher in leukemia stem cells (LSCs) compared with normal HSPCs, so we hypothesize that miR-99a play a role in the HSPCs maintenance and differentiation.We first demonstrated that ectopic expression of miR-99a in cord blood $CD34^+$cells promoted cell proliferation and DNA synthesis. Next, we demonstrated that the percentage of mature granulocytes and monocytes was significantly decreased in miR-99a overexpression group (miR-99a OE) compared with control group after both liquid culture and CFC assay in semisolid medium. Meantime, we also observed that erythrocyte maturation was delayed by flow cytometry analysis during erythroid specific liquid culture. These results suggesting that miR-99a overexpression could partially block myeloid maturation. Furthermore, we scored the LTC-ICs with

limiting-dilution assay. MiR-99a OE group showed higher LTC-IC frequency compared to control group. Simultaneously, there was more $CD34^+$proportion in miR-99a OE group than control group during liquid culture. To elucidate possible mechanism, we performed RNA-Seq analysis and then overlapping the predicted target genes from three databases (miRTarBase, TargetScan and miRanda), and qPCR analysis was performed to validate the potential targets of miR-99a among them.Conclusion:Collectively, our study provides preliminary evidences that miR-99a is crucial for HSPC maintenance, and ectopic expression of miR-99a in HSPCs led to impaired maturation of myeloid lineages.

Keywords: miR-99a, hematopoietic stem/progenitor cells, differentiation

D27

Suppression of Ythdf2-mediated m6A-marked mRNA decay promotes hematopoietic stem cell expansion

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Transplantation of hematopoietic stem cells (HSCs) from umbilical cord blood (UCB) holds great promise for treating a broad spectrum of hematological disorders including cancer, but the limited number of HSCs in a single UCB unit restricts its widespread use. Although extensive efforts have developed multiple methods for ex vivo expansion of human HSCs by targeting single molecules or pathways, it remains unknown whether simultaneously manipulating a large number of targets essential for stem cell self-renewal could be achievable. Recent studies have emerged that N6-methyladenosine (m6A) modulates expression of a group of mRNAs critical for stem cell fate determination by influencing their stability. Among several m6A readers, Ythdf2 is well recognized to promote the targeted mRNA decay. However, the physiological functions of Ythdf2 on adult stem cells are still elusive. Here we show that conditional knockout (KO) mouse Ythdf2 increased phenotypic and functional HSC numbers, but neither skewed lineage differentiation nor led to hematopoietic malignancies. Furthermore, knockdown (KD) of human YTHDF2 led to over 10-fold increase in ex vivo expansion of UCB HSCs, 5-fold increase in colony-forming units (CFUs), and 3- to 4-fold increase in functional UCB HSCs in the limiting dilution transplantation assay. Mechanistically, m6A mapping of RNAs from mouse hematopoietic stem and progenitor cells (HSPCs) as well as from human UCB HSCs revealed m6A enrichment on mRNAs encoding transcription factors critical for stem cell self-renewal. These m6A-marked mRNAs were recognized by Ythdf2 and underwent mRNA decay. In Ythdf2 KO HSPCs and YTHDF2 KD UCB HSCs, these mRNAs were stabilized, leading to an increase in protein levels and facilitating HSC ex vivo expansion. Therefore, our study first elucidates the function of Ythdf2 on adult stem cells maintenance and identifies an important role of Ythdf2 in regulating HSC ex vivo expansion via controlling the stability of multiple mRNAs critical for HSC self-renewal, thus having a strong potential for future clinical applications.

Keywords: Hematopoietic Stem Cell, N6-methyladenosine, HSC ex vivo expansion

D28

Overexpression of the miR-106b~25 cluster promotes cell proliferation and adriamycin-resistance in acute myeloid leukemia

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Background: Leukemia stem cells (LSCs) resistant to chemotherapy are postulated to contribute to the treatment failure and disease relapse. MicroRNAs (miRNAs) are small noncoding RNAs which deregulation have been described in a variety of human cancer types, including acute myeloid leukemia (AML). The miR-106b~25 is a gene cluster including miR-106b, miR-93 and miR-25. We have previously reported that miR-106b~25 is associated with chemotherapy resistance in breast cancer, but its role in leukemia has not yet been elucidated. Here we aim to identify the connection between overexpression of miR-106b~25 and drug resistance in myeloid leukemia.Methods: The expression of miR-106b, miR-93 and miR-25 were examined in enriched LSCs ($CD34^+$ cells) isolated from the bone marrow of 11 newly diagnosed AML patients, respectively, to see whether it was associated with the poor prognosis of AML patients. We also used TaqMan-qPCR assay to detect the expression of miR-106b, miR-93 and miR-25 in the leukemia resistant cell line K562/A02 and HL60/A, compared with their parental cell lines. K562 cells were transduced with lentiviral vectors carrying miR-106b~25, and cell proliferation, drug resistance and colony forming assay were performed to explore the function of miR-106b~25 overexpression in leukemia cells *in vitro*. To investigate the tumor growth and overall survival after drug treatment in *vivo*, we also performed xenotransplantation using miR-106b~25 overexpressed K562 cells in nude mice.Results: Our study showed higher miR-106b expression in enriched LSCs was significantly associated with shortened event-free survival of AML patients.We also found all three miRNAs in this cluster were significantly upregulated in drug resistant leukemia cell lines compared with its parental cell lines.Overexpression of miR-106b~25 cluster promoted cell proliferation, and increased the IC50 of adriamycin and their ability of forming drug-resistant clones in K562 cells. We also observed overexpression of miR-106b~25 cluster promoted tumorigenesis in mice and was significantly

correlated with worse overall survival *in vivo*.Conclusions: Our study suggested that the miR-106b~25 gene cluster may become a potential target related to myeloid leukemia progression and drug resistance.

Keywords: drug resistance, miR-106b~25, myeloid leukemia

D29

Single-cell dissection of long non-coding RNAs pivotal for hematopoietic stem cell development

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Stepwise generation of hematopoietic stem cells (HSCs) from embryonic endothelial precursors is stringently modulated by signaling pathways and transcription factors. Nevertheless, regulatory roles of non-coding RNA remain unknown. Taking advantage of our capacity to capture rare single pre-HSCs and HSCs in vivo, we presented the first long non-coding RNA (lncRNA) landscape of HSC development at single-cell resolution. Combining bioinformatics analysis and functional screening, we identified a set of lncRNAs influencing hematopoiesis in vitro. Using endothelial-specific knockout strategy, we further revealed an lncRNA, namely lnc-preHSC1, was essential for emergence of functional pre-HSCs and HSCs in vivo. Immunophenotypically-defined pre-HSCs from the lnc-preHSC1 mutant embryos were molecularly similar to endothelial cells by single-cell transcriptomic analysis, indicative of the retardation during endothelial-hematopoietic transition in the absence of lnc-preHSC1. Taken together, our study identified lnc-preHSC1 as the first non-coding gene pivotal for embryonic HSC development. The single-cell lncRNAomics map would be a valuable tool for future deciphering the epigenetic regulation of HSC generation.

Keywords: hematopoietic stem cell

D30

Uncovering the Heterogeneity of the Granulocyte-Monocyte Progenitor

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Granulocyte-macrophage progenitors (GMPs) are committed myeloid precursor cells which is well-known to give rise to multiple lineages which include: neutrophils, eosinophils, macrophages and monocytes. However, emerging data suggest that GMPs are highly heterogeneous. To determine this heterogeneity, we employed high-dimensional analyses such as mass cytometry and single-cell RNA sequencing to delineate the phenotype of GMPs. Here, we show that GMPs consists of subsets possessing lineage-specific markers such as FceRa1, Siglec-F, CD115 and Ly-6C. Furthermore, by performing a comprehensive screen of surface marker expression levels within the GMPs, we found new markers that can help in dissecting GMP heterogeneity. Our single-cell RNA-sequencing results also revealed differential gene expression levels according to their lineage specificity. Our study hence suggests a re-definition of the GMP which will help in the better understanding of physiological and pathological myelopoiesis.

Keywords: GMP, myelopoiesis, heterogeneity

D31

Antineoplastic effects and mechanisms of KDM4A inhibitor in acute myelogenous leukemia stem cells

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Acute myeloid leukemia (AML) is a malignant disorder characterized by the abnormal growth and differentiation of hematopoietic stem/progenitor cells (HSPCs), which is common in adults. Lysine demethylase 4 (KDM4) is a member of the JmjC domain containing proteins, which has been found to be related to multiple types of cancer, including AML. Here, we demonstrated that *KDM4A* was highly expressed in both AML cell lines and clinical specimens. Inhibition of KMD4A by siRNA increased the apoptosis of both THP-1 cell and GFP^+ cells from MLL-AF9 leukemia mouse bone marrow. Interestingly, MLL-AF9 GFP^+ cells failed reestablish leukemia *in vivo* when KDM4A was silenced. Furthermore, after two rounds of screening, we identified one compound which showed significant cytotoxicity to AML cell lines, named SD49-7. SD49-7 significantly inhibited the proliferation of THP-1 cells, induced apoptosis, suppressed the cell cycle, and elevated the expression level of CD11b, whose effect are superior to SD70 which was published as a KDM4 inhibitor. We found that SD49-7 decreased expression level of KDM4A, and increased level of H3K9m^2/3 and H3K36me2/3, which were substrates of KDM4A. Limiting dilution transplantation assay showed that the ratio of leukemia stem cells (LSCs) of MLL-AF9 GFP+ cells decreased after the treatment of SD49-7. Moreover, immunodeficient mice transplantation model illustrated that SD49-7 was able to suppress the human leukemia cell *in vivo*. Microarray analysis indicated that *P*53 pathway was involved in the regulation of SD49-7. Our results suggest that KDM4A is important in the development and maintenance in AML, and provide valuable insight into the mechanisms underlying SD49-7-induced cytotoxicity of LSC.

Keywords: AML, KDM4, LSC

D32

The fate decisions of hematopoietic stem cells influenced by multipotent progenitors at clonal level

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Hematopoietic stem and progenitor cells (HSPCs) lodge in specialized microenvironments which control the differentiation and proliferation of hematopoietic stem cells (HSCs). All putative niche cells for HSCs are derived from a nonhematopoietic origin. Recently, several mature blood cells have been implicated in providing feedback and regulating HSC behaviors. However, it remains unknown whether feedback of progenitor cells influences HSCs behaviors. Currently, standard HSC transplantation infuses a mixture of long-term HSCs and short-term hematopoietic progenitor cells (HPCs), such as multipotent progenitors (MPPs). Here we show that MPPs can directly regulate individual HSCs differentiation, and promote more individual HSCs producing T cells population. To better understand how MPPs influence the fate decision of individual HSCs post transplantation, we used one genetic barcoding technology, combining lentiviral delivery of high diversity short genetic barcodes with high-throughput sequencing in order to quantitatively retrieve and track the clonal output from individual HSCs with or without co-transplanted MPPs. Our data showed that increased T cells were due to the combinatorial effects of increasing expanded clones and inhibiting shrunken clones. Furthermore, MPP co-transplantation promoted T-biased lineage differentiation of HSCs. Many other lineage clones changed to T-biased HSC clones. The differentiation exhibited distinct temporal patterns with or without MPP co-transplantation. In conclusion, these results indicate that progenitors derived from HSCs contributes to the HSC niche, directly regulating the behaviors of HSCs.

Keywords: Lineage differentiation, Clonal expansion, Barcode tracking technology

D33

A novel small molecular compound can inhibit telomerase in leukemia cells and efficiently eradicate leukemia stem cells

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The unlimited proliferative potential has already been identified as a major character of cancer cells, thus, telomere maintenance mechanisms have been recognized as a potential therapeutic target. We screened multiple compounds and found out two of them can inhibit telomerase in Hela cells., We examined the effects of one leading telomerase inhibitors IX in multiple leukemia cell lines, and it can selectively eradicate resistant cell lines. The telomere erosion was observed and the expression diversification of Wnt pathway associated proteins were detected in resistant cells treated with IX. Comparing to imatinib, IX is more effective in inducing apoptosis in drug-resistant CML cell lines. These findings indicate that the extension of telomeres is possibly related to Leukemia resistance. We then explored whether this inhibitor can selectively target leukemia stem cells (LSCs). According to in vitro results, treated leukemia cells with IX resulting in significant colony formation suppression, cell cycle arrest and enhanced apoptosis. Furthermore, we use the patient-derived xenograft model to study whether it can delay the growth of primary chronic myelogenous leukemia (CML) cells in vivo. It turns out IX can significantly decrease the engraftment level of leukemia cells. Importantly, this inhibitor didn't show obvious effect on normal CD34+ cells from cord blood.Interestingly, another novel compound we found can also efficiently eliminate leukemia cells but disturb the lengthening of telomeres in a different way from IX. Through the C-circle assay and TRF analyses in U2-OS cells, we suppose the inhibitor may interfere the prolonging of telomere by disturb the alternative lengthening method, which was previously been reported to exit in primary leukemia cells of chronic myeloid leukemia. Our results suggest that aberrant elongation of telomere may play an important role in the development and maintenance in leukemia, and provide the promise of targeting abnormal telomere elongation mechanism for leukemia treatment.

Keywords: telomerase, LSC

D34

Twist1 Promotes MLL-leukemogenesis by Restricting ROS of Leukemia Initiating Cells

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TWIST1 is a transcription factor critical for tumor stemness in various carcinomas. Our previous study has shown that TWIST1 is highly expressed in bone marrow leukemia stem cells (LSCs) of patients with mixed lineage leukemia (MLL)-rearranged acute myeloid leukemia (AML), however, its role in MLL-mediated leukemogenesis remains elusive. To define this, we generated a conditional *Twist1* knockout MLL-AF9 AML mouse model. We found that *Twist1* is indispensable formaintenance and development of MLL-mediated AML and the self-renewal capacity of LSCs. Loss of *Twist1* significantly prolonged the survival of leukemic mice and decreased the leukemia burden by promoting LSCs apoptosis and terminal myeloid differentiation. *Twist1*-deficient LSCs exhibited markedly increased reactive oxygen species (ROS) and impaired capacity of DNA damage repair. Mechanistically, transcriptional profiling of *Twist1*-deficient LSCs revealed changes associated with enhanced *P*53 pathway and oxidative stress responsive genes. Treatment of the *Twist1*-deficient leukemic mice with ROS scavenger restored the maintenance and development of MLL-driven AML. Collectively, our findings uncover a dependency for Twist1 in MLL-leukemogenesis, and reveal Twist1 as a novel potential therapeutic target in this disease.

Keywords: TWIST1, MLL-AF9, ROS

D35

Regulation of HSC fate by ASXL1 through interacting with RNAPII complex in marrow niche

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The Drosophila Asx protein belongs to the enhancer of Trithorax and Polycomb group and functions in both transcriptional activation and repression. Somatic or de novo mutations of *Additional sex combs-like 1* (*ASXL1*) frequently occur in patients with myeloid malignancies or Bohring-Opitz syndrome, respectively. We previously reported that systemic deletion of*Asxl1* leads to severer hematologic phenotypes than the conditional loss of *Asxl1* in hematopoietic cells alone. This indicates that Asxl1 loss in the niche could contribute to the hematopoietic phenotypes *in vivo*.Here we developed an *OsxCre;Asxl1*$^{fl/fl}$ mouse model with conditional knock-out of Asxl1 in the bone marrow stromal cells (BMSCs). Deletion of *Asxl1* in the marrow niche impaired the long-term HSC pool and skewed cell differentiation with a bias to granulocytic/monocytic lineage.RNA-Seq was performed to examine if the dysregulated genes in *Asxl1*$^{-/-}$ BMSCs are associated with the HSC/HPC maintenance. GSEA and GO analyses showed that down regulated genes were associated with cell cycle, cell division, stem cell proliferation, cell surface receptor signaling pathway involved in cell–cell signaling, and mRNA transcription signatures. Up regulated genes were associated with cellular response to interferon-beta, extracellular matrix organization, and cell adhesion. This indicated us loss of *Asxl1* affect phenotype through transcription. Interestingly, the genes critical for the function of BM niche on hematopoiesis, such as *Cxcl1*,*Cxcl2*, and Vcam1, were downregulated in *Asxl1*$^{-/-}$ BMSCs. These data suggest that Asxl1 is required for normal BM niche activity to maintain normal hematopoiesis.Genome-wide mapping of the ASXL1 binding regions showed 44% of ASXL1 binding sites were localized at the promoter regions, suggesting an association of ASXL1 in gene regulation. Genes measured by RNA-Seq were separated to high (75-100%), medium (25-75%), low (bottom 25% of genes with significant output), or silent (genes with nonsignificant output) expression groups, and the density of ASXL1 peaks was indeed associated with gene expression levels.The distribution of ASXL1 at promoter regions was comparable to that of RNA polymerase II(RNAPII) in BMSCs. To examine whether ASXL1 and RNAPII co-localize at the same genomic regions, integrative analyses were performed to assess the genome-wide distribution in BMSCs. The results showed a significantly positive correlation between ASXL1 binding and RNAPII occupancy. ASXL1 promoter binding was highly overlapped with the RNAPII loading. Importantly, analyses of the coverage between ASXL1 and RNAPII bound genes showed a high degree (85.36%) of the overlay. This indicate us ASXL1 may function as a gene regulator through RNAPII in BMSCs.Further immunoprecipitation assays showed that ASXL1 interacted with the core subunit of RNAPII complex, POLR2A,in BMSCs.Enrichment of RNAPII dramatically decreased after *Asxl1*knock out. Traveling ratio (TR) represents the relative ratio of RNAPII density in the promoter

region and the gene body. A substantial shift in TR was observed in *Asxl1*$^{-/-}$ BMSCs compared with WT BMSCs, suggesting a decrease in RNAPII density at the transcribed region. These results indicate an impact of ASXL1 in regulating RNAPII transcription in BMSCs.These data provide a mechanistic basis for ASXL1 functions in the marrow nicheto maintain normal hematopoiesis; and ASXL1 alteration in, at least, a subset of the niche cells induces myeloid differentiation bias, thus, contributes the progression of myeloid malignancies.This work identifies a novel ASXL1 binding complex, RNAPII protein complex, through which ASXL1 loss leads to dysregulated gene expression. Future efforts are warranted to investigate if ASXL1 mutation/dysregulation in the marrow niche of patients contribute to the pathogenesis of myeloid malignancies, which will shed light on identifying novel therapeutic strategies for patients with ASXL1 alterations.

Keywords: ASXL1, niche, RNAPII

D36

Initiation of definitive erythropoiesis before hematopoietic stem /progenitor cells from human pluripotent stem cells

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The development of human erythroid cells has been routinely investigated by using models of adult hematopoiesis, while their initiation during embryonic/fetal stage is largely unknown.We have reported that in a co-culture system with AGM-S3 stromal cells, early erythroblasts derived from human pluripotent stem cells (hPSCs) initially endowed with definitive characteristics but still shared mesodermal and endothelial properties.These early erythroblasts were enriched in $CD34^+GPA^+CD43^+$ ($34^+G^+43^+$) fraction and emerged by day 5 (D5) in co-culture, much earlier than $CD34^+CD45^+$ HSPCs did (generated on D8 in co-culture).Colony forming assay results showed that most of the colonies formed by D7 $34^+G^+43^+$ cells were CFU-E, with very few CFU-GM. However, D10 $34^+G^+43^+$ cells had lower colony forming ability. Even only stimulated with EPO, D7 $34^+G^+43^+$ cells produce large quantity of CFU-E colonies comparable to those with 7 hematopoietic Factors, suggesting they were already erythroid lineage committed. Interestingly, D7 $34^+G^+43^+$ cells could also give rise to endothelial cells in culture. By flow cytometric analysis, these cells both highly expressed erythrocyte-lineage specific markers (CD71, CD41a) and endothelial-related markers (CD31, CD144, CD146), weakly expressed KDR and C-kit, but no expression of CD45, CD184, CD73, CD106, CD36 and CD47. Immunofluorescence staining (IF) showed that 84.7±3.1% of D7 $34^+G^+43^+$ cells were positive for Hb, 32.1±4.4% both positive for Hb and eNOS, and 39±1.3% both positive for Hb and vWF. Besides, addition of VEGF enhanced the production of erythroblasts from $34^+G^+43^+$ cells in serum-free suspension culture. These results demonstrated that $34^+G^+43^+$ cells had both erythroid and endothelial properties. To trace the origin of the $34^+G^+43^+$ cells, we did cell sorting for $34^+KDR^+GPA^-43^-$ or $34^+KDR^-GPA^-43^-$ fractions at D5, and re-cultured with AGM-S3 for 4 days. A large number of $34^+G^+43^+$ cells were generated from $CD34^+KDR^+GPA^-CD43^-$ population, suggesting $34^+G^+43^+$ cells were originated along with embryo definitivehematopoiesis.Single cell RNA-sequencing data showed that $34^+G^+43^+$ cells developed before the emergence of HSPCs. D5 $34^+G^+43^+$ cells had already expressed hemoglobin-related genes, erythroid transcription factors and other erythrocyte-lineage specific markers, suggesting the origin of these erythroblasts was different from HSPCs. In this study, we found a unique embryonic/fetal pattern of erythroblast development, which differs from those derived from adult hematopoiesis. Our study may highlight the understanding of definitive erythropoiesis before the generation of HPSCs during human embryogenesis.

Keywords: erythroblasts, hPSCs

D37

Enhancement of development and maintenance of human ES cell-derived hematopoietic stem cell-like progenitors by overexpression of GATA2

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The multipotential hematopoietic stem/progenitor cells (HSPCs) derived from human pluripotent stem cells (hPSCs) might serve as an ideal substitute for hematopoietic stem cells (HSCs) applicable to clinical use. However, the mechanism controlling maturation and maintenance of hPSC-derived HSC-like cells is still poorly understood, not to mention the reconstitution ability in vivo. GATA2 plays an essential role in the endothelial-to-hematopoietic transition (EHT) and thereafter the generation of HSCs. We have reported an efficient hPSC/AGM-S3 co-culture system to produce robust growth of definitive HSPCs that could be further induced into functionally mature erythrocytes, platelets and other blood cells. By this system, we established a temporally inducible overexpression of GATA2 by doxycyclin (Dox) in human embryonic stem cells (hESCs) to elucidate its functional role in the development, maintenance and differentiation of CD34+CD45+CD90+CD38- HSC-like cells.GATA2 promoted generation of hematopoietic stem / progenitor cells (HSPCs, as CD34+CD43+CD45+ cells) through mesoderm differentiation and EHT. Results revealed that HSPCs increased more than 5.4-fold by Dox induction. Overexpression of GATA2 arrested HSPCs in G0/G1stage in which they sacrificed the cell proliferation rate and myeloid/erythroid differentiation. When released from GATA2 enforcement, these HSPCs could still differentiate into multipotential progenitors that gave rise to functionally mature blood cells such as erythrocytes, granulocyte, mast cells, and macrophages.We further focused on a fraction that mimicking human adult-type HSCs, CD90+CD34+CD45+CD38- cells (HSC-like cells). Enforced expression of GATA2 greatly enhanced the production of HSC-like cells by more than 4.8-fold in co-culture. Along with the maintenance of HSC-like cell property in culture for three weeks, GATA2 also inhibited their differentiation into CD34+CD45+CD38+ progenitor cells. RNA-seq analysis of HSC-like cells with and without Dox indicated 621 genes significantly differed in expression (539 downregulated, 82 upregulated, 6.6-fold, *P* value <0.05). However, the hESC-derived HSC-like cells with or without Dox showed similar expression profile among the HSC specific genes, suggesting GATA2 did not change the HSC properties in this HSC-like cells. Moreover, the GATA2 HSC-like cells decreased in hematopoietic activity because of the arrest in

G0/G1 cell cycle stage.Thus, by inducible GATA2 we established a tracing method through a single gene that conducted the development and maintenance of hESC-derived HSC-like cells. To our data, GATA2 plays dual roles before and after the emergence of CD34+CD43+CD45+ HSPCs along with the maturation of the definitive hematopoiesis: It promotes expansion of mesodermal hemogenic-endothelial progenitors developing into CD34+CD43+CD45+ HSPCs, while thereafter arrests HSC-like cells within G0/G1 cell cycle stages to prevent their further differentiations. Our present study brought evidence that complex and subtle regulation of GATA2 is important in the development, expansion and maintenance of hESC-derived HSC-like cells and may benefit the in vitro production of hPSC-derived HSCs.

Keywords: GATA2, hESCs, Hematopoietic Stem Cells

D38

A role for microglia in hematopoiesis in the embryonic brain

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Along with the aorta-gonad-mesonephros region of the mouse embryo, the head is one of the sites of haematopoietic stem and progenitor cell (HS/PC) development. *In vivo* transplantation of embryonic head region cells shows that HS/PC are localized in the hindbrain and branchial arches (HBA). Fate mapping traces the origins of microglia (brain macrophages) to the yolk sac and recent studies indicate an interaction of primitive macrophages with hematopoietic cells in the embryonic aorta. Whereas, microglia are known to affect neuronal patterning in the embryonic brain, it is unknown whether these cells play a role in head hematopoiesis. Here we characterize microglia in the mouse HBA and examine whether they affect the production of hematopoietic cells in the embryonic brain. We show that microglia in the HBA region are $CD45^{+}F4/80^{+}CD11b^{+}Gr1^{-}$ and express the macrophage-specific *Csf1r-GFP* reporter. In chemokine receptor deficient embryos (*CX3CR1*$^{-/-}$), microglia numbers are reduced and there is a coordinate reduction of haematopoietic progenitors in the HBA. We show in a co-culture system that microglia play a role in endothelial-to-hematopoietic cell transition, boosting HPC output from HBA endothelial cells more than 2-fold. Expression analysis of HBA microglia and endothelial cells implicate signaling through the IL-1 and/or TNFα pathways. The absence of functional HSC in *CX3CR1* mutant and *Csf1r-Cre:RosaDTA* ablation mouse embryos supports a role for microglia in HSC generation in the HBA, thus highlighting a common role for macrophages and inflammatory responses in the hemogenic microenvironment that may shed light on the involvement of microglia in brain neuropathies.

Keywords: microglia, hematopoietic development, brain

D39

Wip1 phosphatase is required for hematopoietic Stem Cell maturation in Mouse Embryos

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Hematopoietic stem cells (HSCs) are generated through a process of multi-step 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−CD41lowc-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.

Keywords: Wip1, Hematopoietic stem cells, maturation

D40

Antioxidant Small Molecule Compounds Enhance Hematopoietic Stem Cells Expansion Ex vivo

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Objective: Through primary screening of 85 antioxidants in small molecule compounds library, we obtained 4 small molecule compounds which facilitated the expansion of human Hematopoietic Stem Cells (hHSCs). Then we further verified and explored the optimum concentration in order to explore their active antioxidant mechanism. Methods: All these small molecules have not been reported in hHSCs expansion. We performed a primary screening with bulk $CD34^+$cells *ex vivo* which could determine the optimum concentration of these compounds. And SR1(1μM) was usedas a positive control. In order to determine the optimum concentration, the absolute number and percentage of $CD34^+$cells and $CD34^+$ $CD49f^+$cells were detected by HTS flow cytometry after 7 days after the treatment of compounds with a series concentrations with bulk $CD34^+$cells *ex vivo*. CFC assay was performed to verify the effect of small molecules on hHSCs self-renewal and differentiation. Results: C2968,D3331,B1753 and B3358 were selected to further analysis after the consideration ofthe concentration, safety and proliferation effect in small molecule compounds library. From the concentration assays, the optimum concentration of C2968,D3331,B1753 and B3358 were 0.5μM, 1.5μM, 1.5μM and 15μM respectively. CFC assay showed that C2968,D3331,B1753 and B3358 could sustainthe multiple differentiation potential of progenitor cells especially multi-potent progenitors. Mean ±SEM values were showed for all experiments(*P<0.05, **P<0.01, ***P<0.005). Conclusion: The antioxidant small molecule compound C2968(0.5μM),D3331(1.5μM),B1753(1.5μM),B3358(15μM)showed good *ex vivo* expansion effect on hHSCs, maintained the self-renewal and multi-lineage differentiation. Through further research on its antioxidant mechanism, they may become a new tool for further understand the antioxidant defense mechanism of hHSCs, and help fundamental research and clinical umbilical cord blood transplantation of hHSCs.

Keywords: antioxidant small molecule compounds, hematopoietic stem cells, expansion

D41

Biphasic modulation of insulin signaling enables highly efficient hematopoietic differentiation from human pluripotent stem cells

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Hematopoietic lineage cells derived from human pluripotent stem cells (hPSCs) hold great promise for the treatment of hematological diseases and providing sufficient cells for immune therapy. However, a simple, cost-effective method to generate large quantities of hematopoietic stem/progenitor cells (HSPCs) is not yet available. In this study, we established a monolayer, chemically defined culture system to induce hematopoietic differentiation from hPSCs in 8 days.RESULTS:We found that insulin-free medium allowed hPSCs to leave pluripotency promptly and preferably enter the vascular lineage. Addition of insulin during the later stage of differentiation was essential for the efficient induction of hemogenic endothelium and the emergence of large numbers of CD34+CD43+ HSPCs, while no insulin condition preferably permits endothelial differentiation. Global transcriptome profiling revealed that HSPCs differentiated using our protocol were similar to embryoid body-derived HSPCs. HSPCs obtained from our differentiation system formed robust erythroid, granulocyte and monocyte/macrophage colonies in CFU assay, and can be induced to generate functional macrophages with robust phagocytic ability. Our results demonstrated that proper manipulation of insulin-mTOR signaling can greatly facilitate HSPC formation. This finding can be further exploited to formulate cost-effective differentiation medium to generate large quantities of cells of desired blood lineages for regenerative medicine.

Keywords: human pluripotent stem cell, hematopoietic differentiation, macrophage

D42

E.coli-elicited acute infection promotes hematopoietic recovery by enhancing granulopoiesis without affecting the long-term reconstitution activity of HSCs

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Background: Radiotherapy and chemotherapy are regular clinical options to treat malignant neoplasms, but leading to neutropenia, anemia or thrombopenia, and high mortality. How to promote hematopoietic recovery is very important. Many studies showed that infection and inflammation could enhance HSPC proliferation, but long-term chronic inflammation reduced the self-renew of HSCs. There is still controversy about whether acute infection and inflammation cause long-term damage to HSCs. Aim: To reveal the potential role of acute inflammation induced by HI *E.coli* in promoting BM hematopoietic recovery and figure out the effect of acute infection and inflammation on the long-term reconstitution activity of HSCs. Methods: HI *E.coli* was used to induce acute peritonitis model. Irradiation models were used to study the hematopoietic recovery. The hematopoietic stem/progenitor cells were mainly analyzed by flow cytometry (surface and cytoplasm markers) and granulocyte-monocyte colony-forming unit (CFU-GM) assay. EdU (5-Ethynyl-2'-deoxyuridine) assay was used to study cell proliferation. Results：1) HI *E.coli* could induce many inflammatory factors such as G-CSF、IL-1β、IL-1α, indicating that HI *E.coli* could induce systematic inflammation. 2) Mice were pretreated with HI *E.coli* before irradiation. It was found that HI *E.coli* could improve the survival rate of irradiated wild type mice. 3) The survival rate of irradiated mice was significantly decreased when the IL-1R signal pathway was inhibited by Anakinra, a receptor antagonist of IL-1β. Moreover, the HI *E.coli* could not improve the survival rate of irradiated IL-1R1 knockout mice. We used HI *E.coli* to pretreat mice 24 hours before 4Gy irradiation and found that the ability of hematopoietic reconstruction of wild type mice was significantly better than IL-1R1 knockout mice, such as increased number of total bone marrow cells, enhanced number of LSK、GMP and BM-neutrophil. Therefore, HI *E.coli*-induced acute infection could promote BM granulocyte hematopoiesis and this protective effect relied on IL-1R1. 4) We cultured different groups of the hematopoietic stem/progenitor cells (HSPCs) in vitro. It was found that IL-1β could be applied directly to HSPCs and promote their proliferation. Also, in vivo results showed that IL-1β had the same effect on HSPCs. 5) We found HI *E.coli*-elicited acute infection could trigger "expansion" of LSK cells and promote granulopoiesis. Although the frequency of functional HSCs in expanded LSK cells was reduced, first and second transplantation assay indicated that HI *E.coli*-elicited acute infection did not affect the long-term reconstitution activity of HSCs. Moreover, HI *E.coli*-elicited acute infection did not alter the number of functional

HSCs in BM, which was very important for long-term hematopoietic recovery of BM after injury. Conclusion: Our research showed HI *E.coli* could promote hematopoietic recovery by enhancing granulopoiesis and relying on IL-1β-IL-1R1 signal. Moreover, the long-term reconstitution activity of HSCs was not impaired. Our research indicated HI *E.coli* vaccination could be a potential therapeutic approach for accelerating BM recovery in neutropenic patients.

Keywords: acute infection, HI E.coli, hematopoietic recovery

D43

Neutrophil Granulopoiesis Under Physiological Conditions By Single-cell RNA Sequencing

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Bcakground: Neutrophils play a key role in host defense against bacterial, viral, and fungal infections. As the first line immune cells to respond to infection, neutrophils are also a crucial component of effector in both innate and adaptive immune systems. Produced in large numbers in the bone marrow, then neutrophils are released into the circulation. The highest percentage of peripheral blood leukocytes are neutrophils, accounting for 50%–70% leukocytes in human. From their generation in bone marrow to their distribution into circulation and inflammatory site is an important but highly complex process, remaining largely unknown. Objective: To explore neutrophils normal granulopoiesis and distribution using single-cell RNA sequencing. Methods: We performed single-cell RNA sequencing on neutrophils isolated from healthy wild type mouse.We used fluorescence-activated cell sorting (FACS) to enrich for Gr1+ leukocytes from bone marrow, spleen and peripheral blood, andc-KIT+ cells from bone marrow. 20,000 individual cells were sequenced by 10x genomics. After quality controls, there were 11917 individual cells retained, including 11397 neutronphils and 520 HSC . We detected about 100,000 reads and 1408 genes per cell on average. Results: We identified 10 clusters by Non-linear dimensional reduction (t-SNE) analysis . Cluster 0 were HSC as expressing CD34. Clusters 1–7 were neutrophils in different stages. G1 (GMP-like) and G2 (Neu-Fcnb) were early stage neutrophils with higher expression levels of Elane, Mpo and Fcnb, Canp, Stmn1,respectively, containing almost all cells from bone marrow. G3-G6 were later stage neutrophils. G3 (Neu-Ltf) expressed Secondary granules genes Ltf, Camp, Lcn2, while G4 (Neu-Ccl6) were mature neutrophils in bone marrow expressing Mmp8, Ccl6, Il1. The two mature clusters, G5 and G6, both contained main cells from peripheral blood and spleen. G5 were naive neutrophils freshly mobilized from bone marrow. Notably,G6 subdivide into two groups, G6a were aged cells and G6b were neu-antivirus for its marker genes enriched in antivirus pathway by gene ontology analysis, indicating G6b may possess antivirus potential that was not mentioned in previous research. There was a small cluster G7 between G3 and G4 which were immature cells from bone marrow and spleen suggesting that bone marrow was not only the place for neutrophils to develop.The exist of G7 in spleen maybe the reason of extramedullary granulopoiesis in inflammation.We next performed several functional genes analyses to validate our map . For granule production, G1 expressed high levels of primary granules genes such as Elane, prtn3 and Mpo. G2

and G3 most expressed secondary granules genes---Ltf, Camp. Tertiary granules genes were expressed in G4-G7. In addition, we analyzed the cell-cycle status using previously reported genes. G0-G2 were actively proliferating cells, but G3-G7 at a relatively quiescent state. EDU in croporation among of neutrophils subset in vitro was consistent with that estimated from our transcriptome analyses. Conclusions: The understanding of neutrophils subgroups is limited by an incomplete morphology characterization. Neutrophils are highly differentiated cells with diverse functions and high heterogeneity. Using deep single-cell RNA analysis, we offered a comprehensive developmental map forneutrophils and our studies also provided new insights on neutrophils contribution by comparing cell from bone marrow, spleen and peripheral blood. We found that PB and spleen had similar neutrophils subpopulation. These information wound help our understanding of normal neutrophils developmental trajectories and provide insight into novel diagnosis and treatment for Neutrophil-associated diseases.

Keywords: neutrophils, granulopoiesis, heterogeneity

D44

Single cell transcriptomic classification reveals a lack of expansion, altered differentiation and a new regulatory lncRNA of hematopoietic stem cells upon transplantation

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Stem cells are a major renewable cell source in tissue regeneration. In the case of hematopoietic stem cell (HSC) transplantation, whether transplanted HSCs undergo a presumed dramatic expansion in order to meet the urgent need of hematopoietic regeneration in myeloablated hosts, has never been rigorously proven owing to unstable surface markers and the retrospective nature of all the functional assays for HSC. Herein, single cell RNA sequencing (scRNA-seq) was applied to first map the hematopoietic system comprehensively during homeostasis. The fate choices of transplanted HSCs were then tracked by scRNA-seq from day 1 to 7 in irradiated recipients after transplantation by the transcriptome-defined cell taxonomy. Unexpectedly, overt expansion of HSC was not observed in this time window. In contrast, the transplanted HSCs rapidly acquired a transcriptional program of lineage-biased multipotent progenitors (MPPs) or occasionally of more

differentiated megakaryocytic/erythroid and myeloid lineages via non-canonical differentiation pathways, which were also functionally validated by *in vitro* single cell colony assay. Moreover, a novel long non-coding RNA for erythroid/myeloid differentiation was revealed and functionally validated. Therefore, this study not only provides an unbiased transcriptome-based cell taxonomy for the mouse hematopoietic system at single cell resolution, but also uncovers fate choices and a functional molecule of transplanted HSCs in myeloablated recipients, thereby having fundamental implications for stem cell therapies.

Keywords: hematopoietic stem cells, multipotent progenitors, single cell RNA sequencing, transplantation, long non-coding RNA

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Npas4l regulates the specification and lineage divergence of hemangioblast in zebrafish

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Vascular- and blood cells are assumed to arise from a common precursor, termed hemangioblast. However, the cellular and molecular mechanism regulating the specification and lineage divergence of this bi-potent progenitor remains elusive. Here, we demonstrate that Npas4l induces the hemangioblast specification from ventral mesodermal cells and further determine the hemato-vascular lineage segregation via different mechanisms during the late gastrulation and early somitogenesis. By using the well-established zebrafish *npas4l* mutant, *cloche*, which lacks both hematopoietic cells and vasculature, we show that loss of *npas4l* results in an abnormal accumulation of ventral mesodermal cells and a severe deficiency of hemangioblast development, while the formation of other mesodermal derivatives, such as pronephros and somites, is not affected. In addition, overexpression of *naps4l* is sufficient to induce the expression of key hemangioblast genes, such as *etsrp*, *scl/tal1*, *draculin*, *lmo2*, *gata1* and *flk1*. Interestingly, temporal overexpression of *npas4l* at late gastrulation fails to induce the upregulation of *gata1*, implying that *gata1* modulates hematopoietic development in an *npas4l*-independent manner in early hemato-vascular development. Taken together, Npas4l is critical for hemangioblast specification and subsequent lineage divergence during zebrafish embryogenesis.

Keywords: Hemangioblast; Npas4l; hemato-vascular lineage divergence; zebrafish

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Global analysis of cell cycle landscape of HSCs during embryonic development

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Fetal liver (FL) is a primary fetal organ where hematopoietic stem cells (HSCs) retain actively cycling to rapidly expand the newly-established HSC pool in mammals. However, the global cell cycle regulatory network of FL HSCs remains incompletely understood. Here, by combining the marker-based of cell sorting and the Fucci system, we decode the cell cycle landscape of FL HSCs from embryonic day (E) 12.5 to E14.5 in mice. Most of HSCs (G1, S, G2/M) are undergoing cell cycle progression, while only 5% HSCs (G0) are quiescent, indicating that cell cycle entry is a major feature of FL HSCs. Moreover, transplantation assay indicates that G0 HSCs display more robust reconstitution ability than that of actively cycling HSCs. Mechanistically, we show that cell cycle regulators including cyclin D, cyclin A, cyclin B and cell cycle inhibitor p16 present a higher expression in HSCs than in endothelial cells, and in particular cyclin D and cyclin B are highly expressed in HSCs at G0 phase, indicating that cell cycle regulators may regulate HSC function. Future investigation is warranted to better understand the regulatory network of cell cycle in FL HSC expansion and function.

Keywords: cell cycle; cell cycle regulator; hematopoietic stem cells; fetal liver

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N-cadherin marks mesenchymal stem cells that maintain reserve hematopoietic stem cells

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Regulation of hematopoietic stem cells (HSCs) by bone marrow (BM) niches has been extensively studied; however, whether and how HSC subpopulations are distinctively regulated under stress by different BM niches remain largely unclear. Here, we functionally distinguished reserve HSCs (rHSCs) from primed HSCs (pHSCs) based on resistance or sensitivity to chemotherapy and further examined their respective BM niches. We found that both pHSCs and rHSCs could support long-term hematopoiesis in homeostasis; however, pHSCs were sensitive to chemotherapy, whereas rHSCs survived chemotherapy, restored the HSC pool, and supported the subsequent regeneration after myeloablation. A whole-mount HSC distribution study revealed that rHSCs were preferentially maintained in the endosteal region that enriches N-cadherin$^+$ bone-lining cells in homeostasis and post chemotherapy. Transcriptome profiling and *in vivo* lineage tracing results characterized N-cadherin$^+$ stromal cells to be functional mesenchymal stem cells, which gave rise to osteoblasts, adipocytes, and chondrocytes during development and regeneration after injury. Finally, we demonstrated that ablation of N-cadherin$^+$ niche cells or deletion of *SCF* from N-cadherin$^+$ niche cells reduced reserve rHSC number and maintenance during homeostasis and post chemotherapeutic stress.

Keywords: N-cadherin; HSC; MSC