Acute myelogenous leukemia is characterized by uncontrolled propagation of abnormal myeloid cells with a limited capacity to differentiate into mature cells. Recent studies have shown that cell fate determinants can modulate differentiation potential during stem and progenitor cell division, although their regulatory roles in hematologic malignancies remain poorly understood. In this study we focus on the RNA binding protein MSI2, which is known to play a role in cell fate determination in metazoans. MSI2 is functionally essential for the development of AML or blast crisis CML and high expression is associated with unfavorable clinical outcomes in AML and ALL. Because MSI2 encodes an RNA binding protein, we hypothesized that MSI2 regulates expression of genes required for aggressive expansion of immature myeloid cells. Through bioinformatics analysis, we identified FLT3 tyrosine kinase as a MSI2 target transcript. RNA immunoprecipitation assays revealed that MSI2 physically interacts with FLT3 mRNA in leukemia cells. Loss of MSI2 functionally impaired clonogenic growth of FLT3-positive AML cells and led to a reduction of FLT3 protein expression without affecting FLT3 mRNA levels. Taken together these data suggest that MSI2 post-transcriptionally regulates FLT3 expression via RNA binding to drive leukemia propagation.

**2017 - EMBRYONIC THYMPOIESIS IS INITIATED BY IMMUNE-RESTRICTED LYMPHO-MYELOID PROGENITOR INDEPENDENTLY OF NOTCH SIGNALING**

Takahiro Itô, Ayana Hattori

University of Georgia

Acute myelogenous leukemia is characterized by uncontrolled propagation of abnormal myeloid cells with a limited capacity to differentiate into mature cells. Recent studies have shown that cell fate determinants can modulate differentiation potential during stem and progenitor cell division, although their regulatory roles in hematologic malignancies remain poorly understood. In this study we focus on the RNA binding protein MSI2, which is known to play a role in cell fate determination in metazoans. MSI2 is functionally essential for the development of AML or blast crisis CML and high expression is associated with unfavorable clinical outcomes in AML and ALL. Because MSI2 encodes an RNA binding protein, we hypothesized that MSI2 regulates expression of genes required for aggressive expansion of immature myeloid cells. Through bioinformatics analysis, we identified FLT3 tyrosine kinase as a MSI2 target transcript. RNA immunoprecipitation assays revealed that MSI2 physically interacts with FLT3 mRNA in leukemia cells. Loss of MSI2 functionally impaired clonogenic growth of FLT3-positive AML cells and led to a reduction of FLT3 protein expression without affecting FLT3 mRNA levels. Taken together these data suggest that MSI2 post-transcriptionally regulates FLT3 expression via RNA binding to drive leukemia propagation.

**2016 - KIT MUTATION ENHANCED HUMAN MEGAKARYO-ERYTHROID RECONSTITUTION IN XENOTRANSPLANTATION SYSTEM**

Ayano Yurino, Takaji Yamamichi

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immunodeficient mice are widely used to reconstitute human hematopoiesis by xenotransplantation. The most commonly used strains are lymphoid-depleted mice established on the NOD background. Previously, we developed a C57BL/6.Rag2nullIl2rgnull mouse line harboring the NOD-specific Sirpa, named the BRGS mouse, of which the xenograft efficiency was equal to that of NRG mice. This simplified mouse is easy to be modified genetically without multiple backcrosses. One of the problems with the current xenograft models is that the engraftment levels of human megakaryo-erythroid cells are still low. It may be because the mouse microenvironment is insufficient to support human hematopoiesis. The BM niches of mice with mutation in the c-kit receptor (Kit) are possibly opened by the fact that these mice can accept donor HSC engraftment without conditioning in syngeneic and allogeneic transplantsations. Thus, we introduced loss-of-function KdWv mutation into BRGS mouse (BRGSK) and evaluated human megakaryo-erythroid reconstitution in this mouse. CD34+ human umbilical cord blood cells were transplanted into sublethally irradiated BRGSK mice. At 8-12 weeks after transplantation, human hematopoietic chimera defined by human CD45+ cells was more than 90% in the BM and this chimeraism was maintained over 24 weeks in BRGSK mice. Furthermore, the percentages of CD33+ myeloid cells in the BM were significantly improved. Surprisingly, CD235a+ erythroid cells including mature erythrocytes and CD41+ platelets were detected in the BM, which can be hardly reconstituted in the previous xenograft models. Confocal immunofluorescence imaging revealed that there were large human erythroid islands in the sternal marrow of recipient BRGSK mice. Our results showed the hematopoietic microenvironment in BRGSK recipients is more suitable for human megakaryo-erythroid development.
Several reports suggest that hemaangioblast could be the common progenitor of both hematopoietic and endothelial cells. Our study indicates that a subpopulation of human mononuclear peripheral blood cells, lacking the expression of lineage commitment antigens (lin-) and expressing CD31, triggered by vascular endothelial growth factor gives rise to vasculature in vitro. These cells, if further instructed by hematopoietic growth factors, are capable of differentiating into erythroid cells. Furthermore, these studies may characterize, in human adult life, a highly enriched populations developed from a common progenitor of both endothelial and erythroid cells. This study, opens the way for innovative projects to demonstrate the existence of such cells within the adult life.

Our preliminary study showed that overexpression of C/EBPα and PU.1 transcription factors could induce the up-regulation of macrophage/hematopoietic cell surface markers in NIH 3T3 cells. Whether the leukemia cell lines can also be reprogrammed into functional macrophage by up-regulating this two transcription factors remains unknown. We first established the overexpression lentivirus vectors. By using lentivirus vector, the stable expression of the C/EBPα, PU.1, CEBPα+PU.1 and only GFP positive Jurkat cell lines were established. FACS showed that half of Jurkat-Pu.1 were upregulated of mac-1, while slightly downregulation of CD3 at day10. CD3 negative cells were about 70.4% and 43.3% for highly CEBPα positive Jurkat and Jurkat-Pu.1+C/EBPα, respectively. Both of them were slightly upregulation of mac-1. While GFP control cells were showed no response. We found that only PU.1 positive cells showed significantly increase in size and granularity when analysed the size and granularity in these genes transfected cells at day 4 and day8. Moreover, we compared the size and granularity of different level fluorescence intensity Jurkat-Pu.1 cells. higher fluorescence intensity means bigger size and more granularity. We extracted RNA from virus infected cells at day 8. We used bone marrow cancer cells as positive control and Jurkat cell as negative control. RT-PCR showed that only Jurkat-Pu.1 group showed myeloid related gene positive like mac-1, CD64, GM-CSFR and CD11b gene, weakly expressed G-CSFR,M-CSFR. T cell related gene Rag1, Rag2 and lck gently downregulated. E2A a transcription factor as lymphocyte showed greatly downregulated, while GATA-3 and Notch-1 slightly decreased. Our results showed that ALL cell line Jurkat can be reprogrammed into CD3 negative and CD11b positive cells. And these cells owned bigger size and more granularity when compared with Jurkat.
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3228 – DECLINED PRESENTATION
PIVOTAL ROLE OF WIP1 PHOSPHATASE IN MATURATION OF EMBRYONIC HEMATOPOIETIC STEM CELLS

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The ontogeny of embryonic hematopoietic stem cells (HSCs) is principally documented to be governed by transcriptional factors and cytokines. However, the regulation of such a dynamic process by other types of molecules remains poorly defined. Here, we report that disruption of Wip1, the wild type P53-induced phosphatase, results in markedly reduced number and defective function of HSCs in E12.5 fetal liver. Most strikingly, adult-reconstituting HSCs are nearly absent in E11.5 Wip1-deficient AGM region. Wip1 deletion leads to significant reduction in number of CD31+CD41+ pre-HSCs (enrichment of HSCs and type 2 pre-HSCs) when that of CD31+CD41+CD45- cells (enrichment of type 1 pre-HSCs) remains unchanged, suggesting blocked maturation of HSCs in vivo. Incubation of type 1 (CD31+CD41+CD45-) and type 2 (CD31+CD45+) pre-HSCs with CCT, the specific inhibitor of Wip1, abrogates and severely impairs HSC formation, respectively. In contrast, the endothelial population (CD31+CD41+CD45- Ter119-) from E10.5 Wip1-deficient AGM region harbors comparable number of hemogenic endothelial cells, albeit with compromised B lymphoid potential in vitro. Quite interestingly, the HSCs with long-term and efficient engraftment potential can be detected in E11.5-12.5 Wip1-/ yolk sac. In Wip1 knockout embryos, expression of hematopoietic related transcriptional factors, such as Runx1 and GATA-2, is significantly reduced in AGM region but up-regulated in yolk sac. Taken together, our study reveals the Wip1 phosphatase is indispensable for specifically the Pre-HSC/HSC maturation in the AGM region.

3229 – DISSECTING ENDOTHELIAL-TO-HEMATOPOIETIC TRANSITION IN HUMAN IPS DIFFERENTIATION WITH SINGLE-CELL ANALYSIS

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We set out to dissect the endothelial-to-hematopoietic transition (EHT) in the human developmental context using single-cell analysis of in vitro differentiation of iPS cells with a WAS-GFP reporter system. Using the newly defined surface phenotype CD43+ CD34+ CD90+CXC4+CD73-, shown to enrich for hemogenic endothelium (HE) in pluripotent stem cell differentiation cultures, we identified day 10 of our protocol as an ideal time-point for EHT single-cell analysis with 23±2.7% (n=41) of CD43+ cells being either HE or Hematopoietic Stem Cell (HSC)-like cells (CD34+CD43+CD73-). We performed RT-qPCR of 398 single CD34+ cells for >90 genes previously identified as being relevant for EHT and HSC function. Unsupervised hierarchical clustering and principal component analysis revealed a continuum of cells progressing from an endothelial to a hematopoietic transcriptional program. The single cells were clustered into 11 groups: groups 2 to 4 display endothelial transcriptional program with notably high levels of KDR, ENG and VE-cadherin, while groups 6 to 8 have hematopoietic signature with expression of key HSC genes such as RUNX1, TALI, LYL1, PBX1, GATA2, GFI1B, MEI1, some of which were downregulated in groups 9 to 11 that instead display upregulation of erythroid/myeloid commitment genes such as GYPA, KLF1 and PRG2. Interestingly, group 5 appeared at the interface of endothelial and hematopoietic lineages, displaying a clear endothelial transcriptional program as well as high levels of key genes associated with hematopoietic development and HSC identity, including WAS-GFP. This data has allowed us to identify a molecule and FACS-based subpopulation of cells undergoing EHT, and to propose strategies for the enrichment of cells within the HE and HSC-like populations that are undergoing or have recently undergone EHT, respectively. Using single-cell analysis of iPS-derived cells, we characterize for the first time, in the human setting and with high level of detail, a subset of cells undergoing EHT.

3230 – DIFFERENTIAL EXPRESSION PROFILES OF MICRORNAS IN THREE DIFFERENT SUBTYPES OF ACUTE PROMYELOCYTIC LEUKAEMIA PATIENTS

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Acute promyelocytic leukaemia (APL) is an M3 subtype of acute myeloid leukaemia (AML). It is also characterized by translocation t(15;17) that generates the PML-RARA fusion gene. Depending on the breakpoint position in the PML gene, three main fusion transcripts usually result. These breakpoints are bcr1 and bcr3 in introns 6 and 3, respectively, and bcr2 in exon 6. The location of bcr1, bcr2, and bcr3 produces fusion transcripts of varying lengths that, as a result, also are referred to as the long (L), variant (V), and short (S) forms, respectively. MicroRNAs are small, non-coding RNA (19-23 nucleotides in size) that may play crucial roles in the pathogenesis of APL. The aim of this study is to describe differences in miRNAs expression in three different subtypes of APL. The microarray platform used to identify differential expression of miRNAs in this study is the Agilent Technology microarray. Following hybridization and data acquisition, data were analyzed using GeneSpring Software V13.0. Targeted genes of particular miRNAs were identified using miRWalk database. PML-RARα transcripts were detected in all patients’ RNA, confirming the clinical diagnosis of APL. We showed that miRNA expression profiling reveals distinctive miRNA signatures that correlate with different subtypes of APL. In this study, we found that miR-125b, miR-181b and let-7f were the most significantly up-regulated miRNAs in L, S, and V subtypes of APL patients, respectively. miR-486 was found to be significantly down regulated in APL patients with L and V subtypes but not in S subtype. These results provide new insights into the dynamic role of miRNAs expression in APL subtypes and maybe useful in developing therapies targeting miRNAs.

3231 – DECLINED PRESENTATION
HEMATOPOIETIC STEM/PROGENITOR CELL CISTROME: ENCYCLOPEDIA OF ELEMENTS GOVERNING HEMATOPOIETIC STEM/PROGENITOR CELL TRANSITIONS

Kyle Hewitt1,2, Kirby Johnson1,2, Xin Gao1,2, Jing Zhang1,2, Duk Hyong Kim3, Prithiva Devadas2, Rajakshma Pratibhasa2, Chandler Zuo3, Rajendra Sanalkumar1,3, Jin-Soo Kim2, Colin N. Dewey4, Sunduz Keles1, Emery H. Bresnick1,3
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Dissecting mammalian genome function is complicated by the extraordinary abundance of cis-elements. At the Gain2 locus, we discovered GATA-2-bound cis-elements with widely variable importance for Gain2 expression and hematopoesis. An essential intronic GATA-2 binding site (+9.5) functions in hemogenic endothelium to promote hematopoietic stem cell (HSC) genesis. By contrast, a distal Gata2 cis-element (−77)
3232 – GUM ARABIC AS FETAL HEMOGLOBIN INDUCING AGENT IN SICKLE CELL ANEMIA; IN VIVO STUDY
Lamis Kaddum\textsuperscript{a}, Imad Fadul\textsuperscript{a,1}Almula, Omer Eissa\textsuperscript{a}, Haydar AbdeleRazig\textsuperscript{a}, Florian Lang\textsuperscript{b}, Amal Saeed\textsuperscript{a,1}
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Background: High levels of fetal haemoglobin (HbF) decrease sickle cell anemia (SCA) severity and leads to improved survival. According to in vivo and in vitro studies, butyrate increases HbF production. Its utilization in clinical practice is hampered, however, by its short half-life. Serum butyrate concentrations could be enhanced by colonic bacterial fermentation of Gum Arabic (GA), edible, dried, gummy exudates from Acacia Senegal tree. We hypothesized that regular intake of GA increases serum butyrate levels, thus inducing HbF production and ameliorating symptoms of sickle cell anemia. Methods: 47 patients (5-42 years) carrying hemoglobin SS were recruited from April 2014 to January 2015. Patients received 30g/day GA for 12 weeks. HbF, blood count and erythropoietin level were measured. The main outcome of interest was the level of HbF after 12 weeks. The secondary outcomes were improvement in clinical and laboratory results. The study was 12 ethically approved by Alneelain University IRB. Results: The study revealed significant increase in HbF level P.V.0.001 [95% CI, 0.43 -1.02], 14 MCV P.V.0.001[95% CI, 2.312 -6.058] and Hematocrit level P.V.0.02[95% CI, 0.124 -1.902]. No significant difference was encountered in platelets count P.V: 0.346 [95% CI, 2.312 -6.058] and Hematocrit level P.V.0.194[95% CI, 8.035-1.68]. 37% of patients experienced minor side effects which resolved within a week. Conclusion: These findings reveal a novel effect of GA, which may be used to foster fetal hemoglobin production.

3234 – CO-CULTURE WITH MESENCHYAL STEM CELLS PREVENTS RADIATION-INDUCED CYTOTOXICITY OF HEMATOPOIETIC STEM CELLS
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Suppression of hematopoietic stem cells and progenitor cells after high-dose irradiation can result in life-threatening injury. Successful care of patients with acute radiation syndrome (ARS) is depending on the ability to stimulate the recovery of surviving hematopoietic stem cells (HSC). The results of currently published investigations describing adult stem cell efficacy in a variety of injury and disease models, including ARS. Recent studies reported that mesenchymal stem cell (MSC) constitutively promote proliferation and differentiation of HSC. In this study, we investigated that co-culture with MSC could overcome radiation-induced toxicity of HSC in vitro system. Umbilical cord blood-derived MSC (CD34\textsuperscript{-} and CD90\textsuperscript{+}) and HSC (CD34\textsuperscript{+} and CD38\textsuperscript{+}) were used. To evaluate the therapeutic efficacy of MSC to injured HSC, HSCs seeded on MSCs (direct) or on MSCs of tranwell (indirect). CD34\textsuperscript{+} cells and apoptotic cells were measured by flow cytometry for evaluating the radiation effects. In addition, alternated proteins were analyzed by ELISA assay. After exposure to radiation (4 Gy), CD34\textsuperscript{+} cells were decreased in direct system, whereas cell proliferation was increased. Indirect culture system maintains proportion of CD34\textsuperscript{+} cells compared with direct system. 6 days following exposure to radiation, apoptotic cell death was increased in single cultured-HSCs (35%). Direct culture system blocked radiation-induced apoptosis (13.5%). However, indirect culture system could not prevent radiation toxicity. These results suggest that HSC and that cell-cell direct contact with MSC is important for blocking radiation toxicity. Thus, MSC directly binds with HSC to overcome radiation injury that could be benefit clinical applications.

3233 – IDENTIFYING MOLECULAR REGULATORS OF HSC MATURATION IN THE MOUSE EMBRYO USING CHIMERIC EMBRYOS AND CRISPR/CAS9
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Hematopoietic stem cell (HSCs), arise de novo from the major arteries of the mouse embryo from E10.5. Thereafter, HSCs colonize the fetal liver (FL) where they proliferate. The first HSCs (pre-HSCs) are enriched in the VE-Cadherin\textsuperscript{CD45\textsuperscript{+}} population of the E11.5 dorsal aorta (DA). By E14.5 however, FL HSCs can be purified using the SLAM-LSK markers (CD150\textsuperscript{+}Lin-Sca1\textsuperscript{-}cKit\textsuperscript{-}) characteristic of adult-type bone marrow HSCs. Importantly, the genetic factors that contribute to pre-HSC to adult-type HSC maturation remain poorly understood. We have therefore initiated studies to identify the transcription factors (TFs) required for adult-type HSC development. By performing RNA-seq using highly purified E11.5 DA pre-HSCs and E14.5 FL SLAM-LSKs, we have identified previously uncharacterized TFs that are upregulated in E14.5 FL HSCs vs. pre-HSCs. To elucidate the role of these TFs, we developed a novel chimeric embryo technique in which gene expression is knocked-down using lentivirus with a GFP reporter. E2.5 embryos are infected with lentivirus targeting TF gene expression, transferred to pseudopregnant females and allowed to develop to E18.5. The effect of gene-knockdown in GFP\textsuperscript{+} cells of the hematopoietic system is then assessed. Transplantation of FL cells from E18.5 chimeric embryos to lethally irradiated adult recipients is also performed to assess the effect of gene knock down on the repopulating ability of HSCs. We have furthered this work by knocking out candidate TF genes in mouse zygotes using CRISPR/Cas9. Knockout of a previously undocumented gene results in up to 10-times fewer SLAM-LSKs in E18.5 embryos and adult mice compared to wild type, providing new insight into HSC development during ontogeny.

3235 – PRESERVATION AND EXPANSION OF UMBILICAL CORD BLOOD (UCB) CD34\textsuperscript{+} HSCS USING RECOMBINANT NM23-H1 PROTEIN
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Previous studies have repeatedly demonstrated that ex-vivo expansion of CD34\textsuperscript{+} cell populations using cytokine cocktails is associated with concomitant differentiation and loss of CD34\textsuperscript{+} positivity. In this study we describe a protocol for expanding umbilical cord blood (UCB) CD34\textsuperscript{+}ve cells whilst maintaining self renewal capacity. We have demonstrated a feedback loop in AML, in which NM23\textsuperscript{-}H1protein secreted by the
primitive CD34+ve blasts binds to the more mature CD34lo/-ve cells, promoting their survival and the release of cytokines that then provide a survival signal for the CD34+ve. Here we investigated whether this mechanism could be used to expand HSCs ex vivo. UCB derived sorted CD34+ve cells were stored at 4°C overnight in serum free media (SFM) before incubating them for 6-10 days in conditioned media generated from CD34- ve cells incubated with elution buffer control or NM23-H1 for 2hrs. Whereas cell numbers and viability declined in control cultures exposed to EB-CM, cells exposed to NM23-CM sustained high viability and entered into modest proliferation over 6-8 days. Despite overnight storage of CD34+ve enriched cell populations at 40C, treatment with Nmn23-CM was associated with an increase in CD34+ve cell number and intensity of CD34 staining. In vitro CFU assays demonstrated statistically significant increases in GEMM-, GM-, G- and M-CFCs and also BFU-E colonies over 3 replatings, indicating prolonged ex-vivo self renewal. The expanded cells also repopulated mice with comparable efficiency to newly purified cells. The expansion of CD34+ve cells was associated with elevated Il6 and Il1beta in the NM23-CM. Substitution of NM23-CM with combined Il1β and Il6 also enhanced ex vivo survival of CD34+ve cells compared to buffer control, however the expansion and cell mean CD34 fluorescence intensity was inferior to that observed with NM23-CM. Together these data demonstrate that NM23- H1 is able to indirectly promote the expansion of CD34+ve HSCs from UCBs whilst preserving their stem-cell like characteristics.

3236 – DECLINED PRESENTATION
ENDOTHELIAL WNT/β-CATENIN BUT NOT WNT SECRETION
TRANSPARENTLY RESTRAINS THE ENDOTHELIAL-HEMATOPOIETIC
TRANSITION DURING DEVELOPMENT
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In mouse mid-gestation embryos, definitive hematopoietic stem progenitor cells are derived directly from a very small proportion of the arterial endothelium, known as hemogenic endothelium. This process is acknowledged as the endothelial-hematopoietic transition (EHT), featured in vivo as the appearance of intra-vascular hematopoietic clusters. Previous studies suggest that the function of Wnt signaling involved in mouse hematopoietic development is stage specific, that at E10.5 the Wnt/β-catenin activity is required to produce hematopoietic cells from endothelial population, but thereafter needs to be downregulated to promote hematopoietic stem cell development. Nevertheless, little is known about the role of Wnt/β-catenin pathway in the emergence of hematopoietic clusters in the embryonic vasculature at early stages (E9.5-E10.0). We show here that genetic deletion of Catenbl gene (coding β-catenin protein) in the endothelium (using Tie2-Cre) leads to an obviously enhanced EHT in the caudal half region at E9.5-E10.0, as evidenced by the 2- to 3-fold increase of CD31+/KFlb+ population by FACS analysis and the increased number of Runx1+ positive intra aortic clusters by immunostaining. Supportively, the transcripts of P2-Runx1 and Gata2 both show up-regulation in the mutant caudal half. To determine whether the endothelium serves as the major derivation of Wnt ligands for the negative effect on EHT, we generate endothelial-specific Wls (coding Wntless protein) deleted mice and find no difference between mutant and control embryos regarding the intra-vascular cluster generation, suggestive of a dispensable role of endothelial Wnt secretion for EHT. These findings collectively indicate a temporally restricted negative effect of Wnt/β-catenin on the EHT at early stages, which will have important implication in understanding the complicated role of Wnt signaling during hemogenic endothelium development.

3237 – PHYTOSPHINGOSINE PROMOTES MEGAKARYOCYTIC
DIFFERENTIATION OF MYELOID LEUKEMIA CELLS
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Phospholipids, in particular those found in intracellular membranes, are known to activate mitogen-activated protein kinase (MAPK). Phospholipids with highly similar structures were unable to induce similar changes indicating that the activity of Phytosphingosine is highly specific. Although Phytosphingosine is known to activate p38 MAPK-mediated apoptosis, the signaling mechanisms involved in megakaryopoiesis appear to be distinct. In sum, we present another model for dissecting molecular details of megakaryocytic differentiation which in large part remains obscure.
ISEH 44th Annual Scientific Meeting
Late-breaking Abstracts, Posters

3239 – THE EFFECT OF MAGNETIC FIELD RF POWER ON THE DIFFERENTIATION ABILITY OF MESENCHYMAL STEM CELLS: NEURONAL DIFFERENTIATION AS EXAMPLE
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Background: The aims of this report include to find out: 1) does MR magnetic field cause damage to the stem cell or its derivatives cell tolerate the energy of radiofrequency used in MRI when we try to tracking the stem cell by MR. Methods and Materials: 1. We examined the mesenchymal stem cell (MSC) and its neuronal derivatives with magnetic field (MF, 3.0T or 7.0T) and also compared them before and after nanoparticle labeling. The nanoparticle we used were iron oxide with dextran coating. 2. We examined the MSC and its neuronal derivatives without/with iron oxide labeling with MF (3.0T or 7.0T) and also scanning the cells as the routine MR examination with radiofrequency (RF) application for about 30 minutes. 3. Comparison the outcome about the MF and RF before and after differentiation. Results: No effect of MF alone, MF+ magnetic nanoparticles, or combination of MF + magnetic nanoparticles + RF on the primary MSC viability in vitro. No effect of MF alone, MF + magnetic nanoparticles, or combination of MF + magnetic nanoparticles, + RF on the MSC derived neuron viability in vitro. After differentiation, neither MF, RF, nor magnetic nanoparticles had adverse effect on the MSC derived neuron. If the MSC exposed to the MF and RF before differentiation, the MSC cannot differentiated appropriately to neuron. Conclusion: The nanoparticles, MF, and RF has no effect on the viability and proliferation of the MSC and its derivative neuron. Before differentiation, exposure to MF and RF damaged the capability of viability and Differentiation of MSC to neuron. After differentiation, the effect of exposure was not significant. Exposure to an appropriate MF and RF, the viability and proliferation of MSC and its derivatives can be increased. During the design of cellular tracking with MR scanner, the adequate timing for examination should be considered before the differentiation from MSC to neuron or even the other cells.

3240 – A HEMATOPOIETIC METHOD OF SEQUENTIAL FLUORESCENCE IN SITU HYBRIDIZATIONS FOR MULTI-GENE ANALYSES AT SINGLE CELL LEVEL
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This work is aimed to establish a single cell multi-gene fluorescence in situ hybridization (FISH) method for hematopoietology analyses. Briefly, 5 colour-fluochromes were used each time to label the different FISH gene probes, then a sequential striping and rehybridization strategy was employed so that up to 20 genes can be detected in a single cell. After each hybridization, the fluorescence signals were recorded in 6 fluorescence filter channels including DAPI, Spectrum Green™, Cy3™, v1, Texas Red, Cy5, and PF-415. A digital automatic relocation procedure was used to ensure that exactly the same microscope field was studied among each stripping and hybridization cycles. In this way, up to 20 genes can be detected within a single normal or diseased cell. In summary, we have developed a practical cytopathology method for analyzing multiple genes at a single cell level. The method can be used for more precise molecular subtyping or clonal evolution analyses of hematological diseases.

3241 – GPR56: A NEW PLAYER IN HSC GENERATION
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Hematopoietic stem cells (HSC) are responsible for the life-long maintenance and regeneration of the adult vertebrate blood system. The first HSCs arise in the mouse embryonic aorta-gonad-mesonephros (AGM) region at E10.5. They are generated through a transdifferentiation process known as endothelial-to-hematopoietic-transition (EHT). Whereas pivotal transcription factors (Runx1 and Gata2) are known to be required for this, we described the whole-transcriptome of EHT only recently. We identified novel candidate players in HSCs arising from EHT. The most highly upregulated gene is the G-coupled receptor 56 (Gpr56), a 7-transmembrane receptor member of the secretin family. We observed that Gpr56 knockdown in zebrafish resulted in highly decreased EHT. Both zebrafish and mouse Gpr56 can rescue the defect, suggesting that its function is conserved across species. In contrast, the characterization of the hematopoietic system of Gpr56 KO mice by Saito et al., precluded any embryonic lethality which is characteristic of pivotal EHT regulators. Recently, Rao et al. have shown residual Gpr56 protein expression in hematopoietic tissues of these mice. We hypothesize that in addition to the residual Gpr56 activity, the lack of embryonic lethality is caused by redundancy with other GPCRs. In fact, our RNAseq and qPCR data show an increase in Gpr97 and Gpr14 expression during EHT, with the highest expression in HSCs (CD31+Ly6A/GFP+cKit+). Moreover, in zebrafish we have demonstrated that Gpr56 morphants can be rescued by both zebrafish and mouse Gpr97 mRNA but not by Gpr14 mRNA.

To assess the role of the three GPCRs during HSC formation a new complete KO model is required. Using the CRISPR/Cas9 technology, we design guideRNAs (gRNAs) targeting Gpr56, Gpr97 and Gpr14. All gRNA/Cas9 combinations have shown biological activity in vitro and we are awaiting the results of their microinjection into one-cell mouse embryos. Future plans include the characterization of the hematopoietic system of triple and single KO animals, with a particular focus on HSC generation and cluster formation at E10.5. These results will shed light on the requirement of Gpr56 in HSC generation for the very first time.

3242 – DECLINED PRESENTATION
EphB4 ENHANCES STROMAL SUPPORT OF HEMATOPOIESIS
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Bone marrow stromal cells (BMSC) are vital components of the hematopoietic stem cell (HSC) niches. BMSC express tyrosine kinase receptor EphB4. We postulated that EphB4 alters the HSC niche microenvironment and in turn affects HSC maintenance. Using a transgenic EphB4 mouse model (EphB4TG), overexpresses EphB4 under the control of collagen type 1 promoter, activated in BMSC, we showed that, EphB4TG mice had increased frequency of LSK and primitive HSC, correlating with increased CFCs and LTC-ICs relative to wildtype (WT) controls. Moreover, EphB4TG BMCs showed a greater capacity to support LTC-ICs relative to WT feeders. Supportive in vitro human studies showed that blocking EphB4 expression of human BMSC by shRNA knockdown decreased LTC-IC output. Bone marrow transplantation studies showed that elevated levels of EphB4 within the BMSC enhanced HSC homing efficiency and hematopoietic reconstitution capacity of transplanted HSC in the irradiated EphB4TG recipients relative to WT controls. Studies examining the expression of crucial hematopoietic supportive factors produced by BMSC revealed that the expression levels of CXCL12, Angiopoietin-1, IL-6 and FLT-3 ligand were significantly elevated in EphB4TG BMSC compared to WT controls. Therefore, EphB4 appears to be a novel component of the HSC niche, mediating BMSC support of hematopoiesis through interaction with its highest affinity binding ligand, ephrin-B2, expressed by human primitive HSC (CD34+CD38-).

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3243 - A DISCOVERY APPROACH USING REGULATORY ELEMENTS OF THE GENE IDENTIFIES NOVEL REGULATORS OF HEMATOPOIESIS
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Endoglin (ENG) is an accessory receptor for TGF-β signalling expressed on FLK1+ mesoderm that is required for normal hemangioblast (HB) early hematopoietic and vascular development. The Eng -/- enhancer together with the promoter (P) has been shown to mediate robust endothelial expression in the mouse embryos. When coupled with the Eng +/+ and Eng +/- enhancers, the construct also targets blood clusters in the dorsal aorta, fetal liver and placenta. To clarify how these tissue specific enhancers of Eng behave at the HB and hemogenic endothelium (HE) stages, we generated ES cell lines by targeting their Hprt loci with various combinations of enhancer-driven reporter constructs. Our results showed that the Eng +/+P/+/-9+/-9 combination preferentially targeted HBs within the FLK1+ cell compartment. By contrast, the Eng -/-P/-t construct was specifically active in HE cells. Importantly, these combinations of regulatory elements showed higher progenitor associated specificity in comparison to surface ENG. We sorted HB and HE fractions marked by these constructs and performed RNA-sequencing to identify genes involved in these cell fate decisions. Zebrafish morpholinos (MO) were used to perturb genes to evaluate their impact on hematopoiesis where no prior knowledge of such existed. Lrp2/orba zebrafish MO lost expression of cmshb and Runx1 expressing cells in hematopoietic regions. LR2 (low density lipoprotein related protein 2) encodes a protein, which is involved in the regulation of embryonic development by signaling through SHH and BMP4. ScIl-null ES cells do not express Lrp2 during differentiation and Lrp2 KO mice exhibit defects in vascular development and reduced AGM hematopoiesis. Taken together, we use regulatory elements as molecular probes to identify previously unknown cell fate determinants.

3244 - ROLE OF THE MEGAKARYOCYTIC NICHE IN LINEAGE-BIASED HEMATOPOIETIC STEM CELLS
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Hematopoiesis is a continuous process of blood-cell production occurring through the orchestrated proliferation, self-renewal and differentiation of hematopoietic stem cells (HSCs). HSCs are regulated by signals generated by cells in localized bone marrow (BM) microenvironments termed stem cell niches. Our recent work showed that megakaryocytes (MK) directly regulate HSC quiescence via CXCL4, a chemokine highly and selectively expressed by MK. We also showed that the association of quiescent HSCs to MK is independent from the association of HSCs to the quiescent arteriolar niche, identified by the pericytic markers NG2 or Nestin. To further elucidate this mechanism of leukemic cell differentiation by Glis2, we compared gene expression of Spi-B stimulated differentiation of AML cells. In Matrigel plug containing K562-HB cells, leukemia cell growth and angiogenesis were evident, while we found neither cell growth nor angiogenesis in Matrigel plug containing K562 cells. Notably, K562-HB-exosomes also induced angiogenesis in Matrigel plug, and K562-exosome did not. In keeping with our previous observation, exosomal mir-135b enhances angiogenesis by targeting factor-inhibiting HF-1 in hypoxic multiple myeloma cells (Blood, 2014 124:3748); exosomal miRNA-mediated angiogenesis play an important role in leukemia cells and BM hypoxia. Further studies are required to understand how soluble factors and/or exosomal contents affect angiogenesis in the BM microenvironment.

3245 - LEUKEMIA CELLS UNDER PERSISTENT HYPOXIA SECRETE EXOSOMES ENHANCING ANGIOGENESIS IN MICE
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Background and Aim: The bone marrow (BM) microenvironment plays a key role in the growth and survival of hematopoietic cells. The BM is known to be highly hypoxic in hematologic malignancies, and that progression of the disease is associated with expansion of hypoxic niches. Emerging evidence suggests communication between leukemia cells and BM microenvironment occurs via exosomes. This study aimed to clarify whether leukemia cells adapted persistent hypoxia like BM niche affects angiogenesis in BM microenvironment. To clarify, forced expression of Glis2 in cMLL-HB cells, leukemia cell line K562 for exosome-generating cells. K562 cells were cultured at 1% O2 for more than 6 months, and we obtained K562 cells that adapted persistent hypoxic condition; we designated as hypoxia-resistant K562 (K562-HR). Exosome secreted from K562 and K562-HR were isolated using a Total Exosome Isolation Reagent (Invitrogen). Exosomal miRNA profiling was done using a TaqMan low-density array (ABI). In Matrigel plug assay, BALB/c nude mice were injected subcutaneously with 400 µL Matrigel containing cells or secreted exosomes. After 3 weeks, the Matrigel plugs were harvested, and stained with anti-CD31 antibody to analyze vessel density. Results and Discussion: There were no significant differences in size and shape of exosomes in K562 and K562-HR. We found a differential exosomal miRNA expression profile between K562 and K562-HR; mir-210 and miR-135b were significantly up-regulated in K562-HR. Exosome-derived miR-135b regulates angiogenesis by targeting factor-inhibiting HF-1 in hypoxic multiple myeloma cells (Blood, 2014 124:3748); exosomal miRNA-mediated angiogenesis play an important role in leukemia cells and BM hypoxia. Further studies are required to understand how soluble factors and/or exosomal contents affect angiogenesis in the BM microenvironment.

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progenitor/stem cells in mice. Therefore, Glis2 may play an essential role in maintenance of hematopoietic progenitor/stem cells. Our results shows that the function of Glis2 in AML cells differs from that in normal hematopoietic progenitor/stem cells, suggesting the possibility that activation of Glis2 may be a therapeutic target for AML.

3247 – THE RELATION OF HEMATOPOIETIC MICROENVIRONMENT AND CHRONIC MYELOPROLIFERATIVE DISORDERS
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Hematopoietic stem cells (HSC) are multipotent stem cells defined by their ability to self-renewal, differentiation, and maintenance of all blood cell types in hematological system. In polycythemia vera (PV), two distinct populations of erythroid progenitor cells have been described indicating the coexistence of a malignant and a nonmalignant population of hematopoietic progenitor cells. In CML, are sustained by leukemic stem cells which like normal HSCs, a range of biological characteristics that enable their long-term survival, and accumulation of clonal myeloid cells that differentiate normally and abnormally. Thus, HSCs source, malignant and nonmalignant hematopoietic progenitor cells will be discussed in the presentation.

3248 – EZH2 LOSS PROMOTES THE TRANSFORMATION OF EARLY T-CELL PRECURSORS
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The Enhancer of Zeste Homolog 2 (EZH2) plays an essential role in the maintenance of a transcriptional repressive state via H3K27me3. EZH2 is generally thought to act as an oncogene in lymphoma by silencing tumor suppressor genes through H3K27me3 modifications. However, loss-of-function mutations of EZH2 have been found not only in myeloid malignancies, but also in Early T-cell precursor (ETP) ALL, suggesting that deregulated EZH2 function is involved in the pathogenesis of ETP-ALL. However its pathogenic underpinnings still remain largely unknown. In order to understand how EZH2 dysfunction contributes to promoting ETP-ALL, we generated a BMT mouse model of ETP-ALL utilizing Ezh2 and p53 conditional knockout mice. At 4 weeks post BMT, we deleted p53 and Ezh2 via administration of tamoxifen. While all p53 KO mice died from CD34+CD25+ DN2-like cells, whose differentiation was markedly compromised beyond the DN2 stage. The pathological T cells with the same characteristics were also detected in the periphery. Thus, Ezh2 loss significantly promotes the development of ETP-ALL in the absence of p53. To understand the underlying molecular mechanism, we performed gene expression analysis of DN1, DN2 and DN3 cells isolated from DKO mice at pre-ALL and ALL stages. As expected, DKO-ALL DN2 cells showed significantly reduced expression of T-cell differentiation regulators, which might account for the propagation of ETP-ALL cells. In conclusion, we have successfully established a mouse model of ETP-ALL utilizing Ezh2 null/p53 null hematopoietic cells, which will be beneficial to decipher the pathophysiology of ETP-ALL.

3249 - MELATONIN SECRETION IN PATIENTS WITH MULTIPLE MYELOMA
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A number of experimental studies showed that melatonin, the main hormone of the pineal gland, exhibits chronobiological, anticancer, pro apoptotic and antioxidant effects. It has been also known that secretion melatonin is disturb in the oncology diseases. The study was aimed to estimate the correlation between melatonin secretion in patients with multiple myeloma. Design and Methods. A total of 21 patients (aged from 44 to 72 years) with multiple myeloma with acute anemia were studied. Control group was represented by 18 patients (aged from 41 to 67 years) with the same stage of multiple myeloma without anemia. Plasma levels of melatonin were estimated by ELISA method. Results. Our results showed that plasma melatonin levels in patients with acute anemia was almost 25 times lower (0.11 ± 0.06 pg/ml) than in control group (2.3 ± 0.38 pg/ml, p<0.01). Conclusion. This observation indicate than exist direct correlation between melatonin plasma levels and development of anemia in the patients with multiple myeloma. Maybe it's possible existence primary regulation erythropoiesis by melatonin in the patients with multiple myeloma, because in normally low plasma level melatonin is not associated to development of anemia, in this research only patients with acute anemia it's observed very low plasma level of melatonin. This is very important result to develop the research in this direction and find the basic cause of correlation between acute anemia and low plasma level of melatonin in patients with multiple myeloma.

3250 - A NEW STRATEGY TO TARGET ACUTE MYELOID LEUKEMIA STEM AND PROGENITOR CELLS USING CHIDAMIDE, A HISTONE DEACETYLASE INHIBITOR
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Leukemia stem cells (LSCs) are responsible for treatment failure and relapse in acute myeloid leukemia (AML). Therefore, development of novel LSCs-targeting therapeutic strategies is of crucial clinical importance to improve the treatment outcomes of AML. Histone deacetylase (HDAC) inhibitors have shown potent and specific anticancer stem cell activities in preclinical studies. Chidamide, a novel benzamide-type selectively HDAC inhibitor, has been reported to induce G1 arrest and apoptosis in the relatively mature progenitor population, whereas its effect on primitive LSCs has not been clarified. In this study, we demonstrated that chidamide specifically induces apoptosis in LSC-like cells and primary AML CD34+ cells in a concentration- and time-dependent manner. Our further molecular mechanistic study uncovered that chidamide induces LSCs death by...
activation of reactive oxygen species (ROS). It compromises the mitochondria membrane potential, modulates anti-apoptotic and pro-apoptotic proteins in BCL2 family and activates caspase-3 leading to PARP degradation. Meanwhile, chidamide activates CD40 and modulates its downstream signaling pathways, JNK and NFκB. The results of this study suggest that chidamide may be a novel LSC-targeting agent for AML therapeutics.

3251 – MAGNETIC RESONANCE IMAGING MEY TRACE IMPLANTED HUMAN MESENCHYMAL STEM CELL IN MOUSE BRAIN
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Fluorescence application is a huge step for cell tracing especially in the in vitro circumstances. However, the penetration depth of fluorescence in vivo limits its application. Magnetic resonance imaging (MRI) has unlimited penetration depth diction capability and free of ionizing radiation that is ideal for living organism observation. Stem cell therapy has high potential to treat neurodegenerative disorder. However, there is no good way to trace implanted cell. Here, we use OATP1B3 as a reporter to trace implanted cell. OATP1B3, a transporter protein, can enhance MRI signal by absorbing Gd-EOB-DTPA as contrast medium is used here as a reporter gene for tracing brain-injected human mesenchymal stem cell (hMSC). We first transduced OATP1B3 into U87MG, glioblastoma cell, and implanted it to mice brain. The primovist was given to xenograft mice by tail vein injection. The MRI signal could be traced clearly. Further, we transduced OATP1B3 into hMSC. The overexpression of OATP1B3 in hMSC didn’t affect cell viability and differentiation. Also, OATP1B3 overexpressed hMSC was implanted to mice brain and can be detected at different time frame by T1 weighted sequences under 7 Tesla MRI. From above results, we could know that OATP1B3 application was an effective tool for cell transplantation monitor.

3252 – DISULFiram COMBINED WITH COPPER INDUCES LEUKEMIA STEM AND PROGENITOR CELLS APOTOPSIS THROUGH TNF-α/ROS PATHWAY
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Leukemia stem cells (LSCs) have been a major hurdle for the success of acute myeloid leukemia chemotherapy. Thus new drug targeting LSCs is urgently needed. Disulfiram (DS) is an anti-alcoholism drug that combined with Copper(Cu) has been indicated to show the antitumor activity in a wide range of cancer cell lines. In this study, the ability of DS/Cu to induce apoptosis in leukemia stem and progenitor cells was investigated. Leukemia stem and progenitor cells were sorted from KG1a cells and then were subjected to different treatments and flow cytometry and Western blot or RT-PCR, intracellular ROS measurement were used to determine apoptotic status and ROS level. The apoptotic proportion of CD34+CD58+KG1a cells exposed to DS was (11.87±1.30)% (P=0.01), while (27.43±1.65)% to DS/Cu (P<0.01), treatment using DS/Cu significantly increased the percentages of apoptotic cells compared with the treatment with DS alone (P=0.00). DS and DS/Cu could induce ROS accumulation by 1.39±0.115 fold and 2.81±0.109 fold, respectively (P=0.03, P<0.01). However, cell apoptosis induced by DS/Cu were inhibited by pre-treatment of ROS inhibitor (NAC) (27.43±1.65)% vs. (12.37±0.85)% (P<0.01). The mRNA level of TNF-α, CD40, TNFRSF1B, TNFRSF1B, HRK were up-regulated by treatment of DS and DS/Cu (P<0.05), which was more extended by DS/Cu (P<0.05). Pre-treatment of NAC abolished CD40, TNFRSF1B, TNFRSF1B, HRK expression induced by DS/Cu (P<0.05), whereas, these effects were not observed in TNF-α expression (P=0.73). To further confirmed the role of TNF-α, ROS accumulation were inhibited by pre-treatment of neutralization antibody TNF-α mAb (2.78±0.25 fold vs. 1.28±0.17 fold, P<0.001). In conclusion, our data demonstrate that DS/Cu induced LSCs apoptosis through TNF-α/ROS pathway.

3253 – ROLE OF INTRINSIC PATHWAY IN DS/Cu-INDUCED APOPTOSIS IN HUMAN B-LEIGNEAGE ACUTE LYMPHOBLASTIC LEUKEMIA
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Background: So far, the therapeutic regimen of adult B-ALL often results in refractory and relapse. So it is urgently needed to explore new regimen. Currently, high-throughput screening and optimization of candidate compounds are mainly used to develop anti-cancer drugs. However, these strategies are often time-consuming. Disulfiram(DS) has been used clinically as an anti-alcoholism drug for over 60 years. Recent studies disclosed that DS/Cu was cytotoxic against multiple solid cancers and hematological malignancies, but its effects on B-ALL remain unclear. In this study, we studied the effects of DS/Cu on B-ALL cells and its molecular mechanism.

Results: Firstly, CCK8 assay indicated that DS/Cu markedly inhibited Nalm6 proliferation. Secondly, colony-forming assay showed that DS/Cu abolished the clonogenicity of Nalm6. Thirdly, FACS analyses revealed that DS/Cu induced apoptosis of Nalm6, as well as primary B-ALL cells. We additionally analyzed the relationship between clinical parameters of B-ALL patients-including age, WBC counts, immunophenotype, cytogenetics, risk grade and Ph chromosome–with the efficacy of DS/Cu. The apoptotic ratio from Pro-B and genetic abnormality subgroup higher than the corresponding group. To decipher its cytotoxic mechanism, JC-1 staining was done and the results showed that DS/Cu greatly reduced the mitochondrial membrane potential in Nalm6 and primary B-ALL cells. Consistently, Western Blot analysis showed that DS/Cu downregulated the expression of Bcl2 and Bcl-XL, and activated caspase-3 and its substrate PARP.

Conclusion: Our results showed that DS/Cu significantly inhibited proliferation and clonogenicity, and meanwhile induced apoptosis of B-ALL cells. And the intrinsic apoptotic pathway might be the mechanism of DS/Cu-induced apoptosis in B-ALL cells.

3254 – HYPERACTIVITY LEADS TO MYELOID AND LYMPHOCYTIC MALIGNANCIES IN ZEBRAFISH
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The c-MYB transcription factor is a key regulator of hematopoietic cell proliferation and differentiation, and dysregulation of c-MYB activity often causes hematological...
disorders. However, its pathogenetic role remains largely unknown. In this study, we characterize a c-myb-gfp transgenic zebrafish harboring c-myb hyperactivity (named c-myb<sup>hyper</sup>). We found that c-myb<sup>hyper</sup> zebrafish exhibits myelodysplastic syndrome (MDS)-like phenotypes from the embryonic stages to adulthood. Remarkably, a portion of c-myb<sup>hyper</sup> fish develops acute myeloid leukemia (AML)-like or acute lymphoid leukemia (ALL)-like disorders with age, with the infiltration of leukemic blasts in the circulation and other tissues including gill, muscle, liver, eye, and the central nervous system. These phenotypes resemble human myelodysplastic syndrome, AML and ALL, and anti-leukemia chemotherapy treatment can reduce the hematopoietic abnormalities in c-myb<sup>hyper</sup> embryos. Our study reveals that c-myb<sup>hyper</sup> zebrafish provides a valuable animal model for studying the molecular mechanisms underlying c-Myb-associated leukemogenesis and for anti-leukemic drug screening.