



Institute for Regenerative Medicine

**HEMATOPOIETIC STEM CELLS:
BIOLOGY, DISEASE, AND THERAPY**

TUESDAY, MARCH 9, 2021 ♦ 10 AM- 5 PM EST



PennIRM

Institute for Regenerative Medicine

About the IRM

The [Institute for Regenerative Medicine \(IRM\)](https://irm.med.upenn.edu/) at the University of Pennsylvania is a community of experts working to create a future where diseases are cured with cellular solutions. Our mission is to understand how cells and tissues are formed and to apply that knowledge to next generation diagnostics and therapies. We are proud to feature faculty from 5 different Penn Schools, the Children's Hospital of Philadelphia (CHOP), and the Wistar Institute.

IRM Symposia

The IRM typically hosts 2 major symposia each year, one in the spring and one in the fall. These events are a chance to bring together IRM researchers and experts from around the world to discuss the latest topics in regenerative biology and medicine. Recent symposia topics have included the science and ethics of models of early human development, cell & tissue organization engineering, and cellular plasticity. For information on future IRM events, please visit our website (<https://irm.med.upenn.edu/>).

Symposium Organizers

- Ivan Maillard, MD, PhD, is a Professor of Medicine at the University of Pennsylvania and Co-Director of the IRM's Hematopoietic Stem Cell Program.
- Wei Tong, PhD, is an Investigator at the CHOP Research Institute and a Professor of Pediatrics at the University of Pennsylvania. Wei serves as Co-Director of the IRM's Hematopoietic Stem Cell Program.

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HEMATOPOIETIC STEM CELLS: BIOLOGY, DISEASE, AND THERAPY

TUESDAY, MARCH 9, 2021 ♦ 10 AM- 5 PM EST

Schedule

10:00-10:05 **Welcome**
Ken Zaret, PhD, IRM Director
Ivan Maillard, MD, PhD, IRM Hematopoietic Stem Cell Program Co-Director

10:05- 11:05 **Keynote: Sean Morrison, PhD (University of Texas Southwestern)**
Stem cell niche regulation: beyond growth factors

Session 1 **Hematopoietic Stem Cell Biology**
Chair: Vikram Paralkar, MD

11:05-11:35 **Jeff Magee, MD, PhD (Washington University)**
Neonatal HSC ontogeny and the origins of pediatric AML

11:35-12:05 **Daniel Lucas, PhD (Cincinnati Children's Hospital)**
Spatial organization and local regulation of hematopoiesis

12:05-12:35 **Camilla Forsberg, PhD (UC Santa Cruz)**
Blood for life

12:35-12:45 **Trainee Lightning Talks**

12:45- 1:45 **Lunch Break and Poster Session**

Session 2 **Bone Marrow Failure Syndromes**
Chair: Peter Kurre, MD



1:45-2:15 **Vijay Sankaran, MD, PhD (Boston Children's Hospital)**
Genetic studies of human hematopoietic stem cells

2:15-2:45 **Akiko Shimamura, MD, PhD (Boston Children's Hospital)**
Mechanisms of clonal evolution in bone marrow failure

2:45-3:15 **Daria Babushok, MD, PhD (University of Pennsylvania)**
Mechanisms of immune-mediated bone marrow failure

3:15-3:30 **Break**

Session 3 **Hematopoietic Stem Cell-based Gene Therapy**
Chair: Wei Tong, PhD, IRM Hematopoietic Stem Cell Program Co-Director



3:30-4:00 **Paula Rio, PhD (CIEMAT/CIBERER-ISCIII, Madrid)**
Gene therapy for Fanconi anemia

4:00-4:30 **Saar Gill, MD, PhD (University of Pennsylvania)**
Genetic engineering of human HSCs to induce vulnerabilities to immunotherapy

4:30-5:00 **Stefano Rivella, PhD (Children's Hospital of Philadelphia)**
Gene therapy using HSC: successes and challenges

5:00 **Closing Remarks**
Wei Tong, PhD, IRM Hematopoietic Stem Cell Program Co-Director

Daniel P. Brown ^{1,2}, Saar I. Gill ^{2,3}

Affiliations

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Therapeutic Reversion of Pathogenic Point Mutations with Adenine Base Editing

Acute myeloid leukemia (AML) is a poor-prognosis hematological malignancy characterized by dysregulated cell proliferation and differentiation. Approximately 20% of AML cases are linked to isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) missense mutations that elicit the production of the oncometabolite 2-hydroxyglutarate (2-HG). 2-HG contributes to AML pathogenesis by directly inhibiting TET family DNA demethylases and subsequently silencing tumor suppressor genes. IDH1 was recently implicated as disease-initiating in some patients with AML. Therapies that target mutant IDH1 are of great interest, and the FDA recently approved ivosidenib for relapsed AML. Nonetheless, the drug as monotherapy is not curative.

The most common IDH1 mutation is a guanosine for adenine substitution at codon 132. We sought to reverse this pathogenic mutation using adenine base editors (ABEs), which are synthetic Cas9- fusion proteins that enact locus-specific adenine-to-guanine base substitutions without inducing double strand breaks, thereby reducing genotoxicity and unintended genomic alterations. We reasoned that this approach would selectively target mutant IDH1 while sparing the endogenous gene, yielding permanent reversion of the mutation with minimal off-target effects. We tested 8 guides and delivered the base editor ABEmax using electroporation to Molm14 cells. We found 75% reversion of R132H, and we are now quantifying 2HG using high-performance liquid chromatography. In future work we will develop delivery system involving lipid nanoparticle-encapsulated ABE machinery for therapeutic reversion of pathogenic point mutations in vivo.

Understanding Epigenome of Hematopoietic Stem cells

Author: JIGISHA CHANDRA - Bachelors of Pharmacy Honours, Birla Institute of Technology and Sciences, Pilani (BITS Pilani), Pilani, India

Abstract

Hematopoietic stem cells have potential to differentiate into mature cells of both myeloid and lymphoid lineages because of mechanisms of self renewal . Hematopoietic stem cells and cells of myeloid and lymphoid lineage all have the same genome but they have different structure and function. What is the reason behind this? This is because of the difference in their expression of epigenome of cells of various lineages . Their gene expression is governed by a lot of epigenetic markers like DNA Methylation, post-translational histone modifications, nuclear architecture, noncoding RNAs etc. Various epigenetic markers are responsible for expression or repression of an individual or sets of genes. This helps the Hematopoietic stem cells to differentiate into cells of various lineages. This article will focus on how with the help of understanding epigenetic machinery we can understand the development and differentiation of hematopoietic stem cells into various other cells. Also it will help us understand how change in epigenome can lead to change in the architecture of cells. Having an understanding of the above mentioned points, will help us to understand clinical applications - such as diagnosis , prognosis and therapy for disorders related to Hematopoietic stem cells.

Keywords-Hematopoietic stem cells, self renewal, DNA Methylation, post-translational histone modifications, nuclear architecture, noncoding RNAs, as diagnosis , prognosis and therapy for disorders related to Hematopoietic stem cells.

A bioengineered bone marrow ECM niche model to support long-term HSCs *in vitro*

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Hematopoietic stem cells (HSCs) have the ability to regenerate the entire blood and immune system¹, as such, they hold enormous clinical potential. *In vivo* they are supported by mesenchymal stromal cells (MSCs) in a specialised microenvironment that provides physical and functional regulatory cues, termed the *bone marrow (BM) niche*. Once HSCs are removed from the niche their ability to self-renew is lost quickly, cells rapidly proliferate and spontaneously differentiate away from the long-term reconstituting (LT-HSC) phenotype in a matter of days, rendering them clinically ineffective². Here, we have developed a system to recapitulate properties of the BM niche microenvironment to support LT-HSCs *in vitro*. Using poly (ethyl acrylate) (PEA) to promote material-driven unfolding of the extracellular matrix protein fibronectin (FN), we tether BM niche associated growth factors (GF) (e.g. BMP-2) to the exposed GF binding domain on FN³. Then by incorporating a collagen type I hydrogel in the stiffness range of the bone marrow (~0.1 kPa)⁴, we were able to demonstrate maintenance of a niche-like phenotype in MSCs. This included expression of nestin, a key niche stromal marker⁵, and production of HSC maintenance cytokines CXCL12 and SCF. Upon co-culture with HSCs, we found that this niche-like system is able to support LT-HSCs in culture. This material-based system offers a platform for investigation into fundamental mechanisms of stem cell regulation, such as stem cell metabolism, as well as a more humanised model for genotoxicity screening of drugs, small molecules and cellular therapies.

Acknowledgements

We gratefully acknowledge support of EPSRC grant EP/P001114/1 and the technical assistance provided by Carol-Anne Smith.

References:

1. Jagannathan-Bogdan, M. & Zon, L. I. *Development* 140, 2013.
2. Boitano, A. E. et al. *Science*. 329, 2010.
3. Cheng, Z. A. et al. *Advanced Science*. 1800361, 2018.
4. Jansen, L. A., et al. *Journal of the Mechanical Behavior of Biomedical Materials*. 50, 2015.
5. Méndez-Ferrer, S. et al. *Nature* 466, 2010.

Different patterns of chromatin opening by hematopoietic pioneer factors

Megan A Frederick^{1,2}, Meilin Fernandez Garcia², Gregory Donahue², Edgar Luzete Monteiro², Kenneth S Zaret²

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Pioneer factors can elicit local exposure of a nucleosome within chromatin and ultimately recruit co-regulators and remodelers to yield open chromatin sites seen *in vivo*. Yet how different pioneer transcription factors initially expose a targeted nucleosome in compacted chromatin structures is unclear. We used nucleosome arrays *in vitro* with a central nucleosome that can be targeted by the hematopoietic Ets factor PU.1 and the bZIP factors C/EBP α , and C/EBP β . Each class of factor can elicit targeted nucleosome exposure on linker histone-compacted arrays, but with different hypersensitivity patterns, as discerned from long-read sequencing. The DNA binding domains (DBDs) of PU.1 and C/EBP α are sufficient for mononucleosome binding but are much less efficient than the full-length proteins, which can function cooperatively, in opening compacted chromatin. Thus, pioneer factors use their DBD to bind nucleosomes and other domains to elicit distinct patterns of target nucleosome exposure within compacted chromatin *in vitro*. Finally, nucleosome exposure by PU.1 enables further chromatin remodeling by a SWI/SNF complex. Together our data give a mechanistic view of how transcription factors cooperate to disrupt chromatin structures to initiate DNA accessibility to additional regulatory factors.

Title:

A novel role of let-7b/ LIN28B/IMP-1/IGF-II axis as a molecular regulator of megakaryocyte development during aging

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Abstract:

Background: microRNAs (miRNAs) have been shown to play crucial role in the regulation of stem-cell differentiation in normal as well as malignant haematopoiesis. Neonatal cord blood (CB) cells are promising alternatives to adult bone marrow (PB) in cellular therapies. CB cells have potential to develop into specialized cells, such as erythrocytes or megakaryocytes (MK). MKs are responsible for production of platelets and thrombocytopenia (low platelet count) is common among sick infants. We hypothesize that developmental differences between CB and PB-MKs contribute to the vulnerability of neonates to develop severe thrombocytopenia and miRNA role in megakaryocytopoiesis is unknown.

Methods: We cultured human CB and PB-derived CD34⁺ cells in the presence of thrombopoietin for 14 days and collected cultures expressing >90% CD41⁺ by flow cytometry. miRNA was prepared using the miRNeasy mini kit (Qiagen) and expression analysis of 88 miRNAs was performed using an array (Qiagen). Web-based computational approaches were used for putative target prediction and the protein levels were detected using western blot analysis.

Results: Out of 88 miRNAs involved in stem cell development, let-7b was the only miRNA down regulated (~10-fold) in neonates compared to adult megakaryocyte (MK), and the levels of let-7b was differentially expressed in all stages of MK development (progenitors to maturation). We show that the low levels of let-7b in fetal and neonatal MK progenitors cause their relatively high proliferative rate by allowing an up-regulation of insulin-like growth factor II mRNA-binding protein 1 (IMP-1) and insulin-like growth factor II (IGF-II), and that these factors contribute to the marked hyperproliferative response of fetal, but not adult MK progenitors.

Conclusions: The present study shows functional significance of LIN28B/IMP-1/IGF-II axis during megakaryocyte development. Therefore, it could be a potential target in neonatal thrombocytopenia and other platelet disorders.

***LNK(SH2B3)* Inhibition Enhances Human Fanconi Anemia Hematopoietic Stem and Progenitor Cell Function**

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Hematopoietic stem cell transplantation (HSCT) remains the only curative treatment for a variety of hematological diseases. Allogenic HSCT requires hematopoietic stem cells (HSCs) from matched donors and comes with cytotoxicity and mortality. Recent advances in genome modification of HSCs have demonstrated the possibility of using autologous HSCT-based gene therapy to cure monogenic diseases, such as the inherited bone marrow failure syndrome Fanconi Anemia (FA). However, insufficient HSC numbers and our inability to reliably expand HSCs, results in delayed hematopoietic recovery and increased risk of graft failure. We and others previously identified the adaptor protein Lnk(Sh2b3) as a critical negative regulator of murine HSC homeostasis. However, whether LNK(SH2B3) controls human HSCs has not been studied. Here, we demonstrate that depletion of LNK via lentiviral expression of miR30-based short hairpin RNAs (shRNAs) resulted in robust expansion of transplantable human HSCs that provided balanced multilineage reconstitution in primary and secondary mouse recipients. Importantly, LNK depletion enhanced cytokine mediated JAK/STAT activation in CD34⁺ hematopoietic stem and progenitor cells (HSPCs). Moreover, we demonstrate that LNK depletion ameliorated

functional defects in primary HSPCs associated with FA. In xenotransplant, engraftment defects of FANCD2-depleted FA-like HSCs were markedly improved by LNK inhibition. Finally, targeting LNK in primary bone marrow HSPCs from FA patients enhanced their colony forming potential *in vitro*. Together, these results demonstrate the potential of targeting LNK to expand HSCs to improve HSCT and HSCT-based gene therapy.

Title: Constrained chromatin accessibility in PU.1-mutated agammaglobulinemia patients.

Carole Le Coz¹, David N. Nguyen², Chun Su¹, Michael V. Gonzalez¹, Andrew D. Wells¹, Alex Marson², Gregory M.K. Poon³, and Neil Romberg¹

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Background: The pioneer transcription factor (TF) PU.1 controls hematopoietic cell fate by decompacting stem cell heterochromatin and allowing non-pioneer TFs to enter otherwise inaccessible genomic sites. PU.1 deficiency fatally arrests lymphopoiesis and myelopoiesis in mice but human congenital PU.1 disorders have not previously been described. We studied six unrelated, agammaglobulinemic patients each harboring a heterozygous mutation (four de novo, two unphased) of *SPI1*, the gene encoding PU.1.

Rationale: To determine if and how *SPI1* mutations cause PU.1-mutated agammaglobulinemia (PU.MA).

Methods: PU.MA subject DNA, peripheral blood and bone marrow samples were analyzed with whole exome sequencing, CITE-Seq, flow cytometry and immunohistochemistry. The transcriptional capacity, nuclear localization and DNA binding of PU.1 mutants were assessed with reporter lines, confocal microscopy, electrophoretic mobility shift assays. Hematopoiesis and hematopoietic chromatin accessibility were modelled *in vitro* using genetically edited RS4;11 pro-B cell lines and human hematopoietic stem cells (HSCs).

Results: Affected patients lacked circulating B-cells and possessed few conventional dendritic cells. Introducing disease-similar *SPI1* mutations into human hematopoietic stem and progenitor cells impaired early *in vitro* B-cell and myeloid-cell differentiation. Patient *SPI1* mutations encoded destabilized PU.1 proteins unable to nuclear localize or bind target DNA. In PU.1-haploinsufficient pro-B cell lines, euchromatin was less

accessible to non-pioneer TFs critical for B-cell development and gene expression patterns associated with the pro to pre-B-cell transition were undermined.

Conclusions: Our findings molecularly describe a novel form of agammaglobulinemia and underscore PU.1's critical, dose-dependent role as a hematopoietic euchromatin gatekeeper

**HectD1 controls hematopoietic stem cell regeneration by coordinating ribosome assembly
and protein synthesis**

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Abstract

Impaired ribosome function is the underlying etiology in a group of bone marrow failure syndromes called ribosomopathies. However, how ribosomes are regulated remains poorly understood, as are approaches to restore hematopoietic stem cell (HSC) function attributable to defective ribosome biogenesis. Here we uncover a previously-unappreciated role for the E3 ubiquitin ligase HectD1 in regulating HSC function via ribosome assembly and protein translation. *Hectd1*-deficient HSCs exhibit a striking defect in transplantation ability and ex vivo maintenance, concomitant with a reduced protein synthesis and growth rate under stress conditions. Mechanistically, HectD1 ubiquitinates and degrades ZNF622, an assembly factor for the ribosomal 60S subunit. *HectD1* loss led to an accumulation of ZNF622 and the anti-association factor eIF6 on the 60S, resulting in 60S/40S joining defects. Importantly, *Znf622* depletion in *Hectd1*-deficient HSCs restored ribosomal subunit joining, protein synthesis, and HSC reconstitution capacity. These findings highlight the importance of ubiquitin-coordinated ribosome assembly in HSC regeneration.

Title: Improving *in vitro* bone marrow niches for stem cell maintenance

Authors and Affiliations: Chanelle A. S. McGuinness*, Yinbo Xiao*, Steve Swioklo[§], Manuel-Salmeron-Sanchez*, Hannah Donnelly*, Matthew J Dalby*

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Abstract:

The therapeutic potential of hematopoietic stem cells (HSCs) is well known. However, harnessing their full potential is limited since they quickly lose their stem cell properties *in vitro*. In addition, mesenchymal stromal cells (MSCs) fail to adequately support HSCs *ex vivo*. These limitations in *ex vivo* culture of stem cells are believed to be due to lack of regulatory signals that are normally supplied to HSCs and MSCs within the bone marrow niche. Biomaterials have been of great interest to mimic aspects of the 3D environment of tissues. In our laboratories, recent work demonstrated how MSCs can be maintained and supported using low stiffness collagen hydrogels on PEA coated surfaces functionalised with fibronectin and growth factor BMP-2 *in vitro* to mimic aspects of the bone marrow niche. This 3D niche model could support MSC niche characteristics including Nestin, SCF and CXCL12 expression and promote maintenance of long-term HSCs. However, the use of collagen hydrogels brings limitations to this model since they are animal derived, difficult to modify and demonstrate batch-to-batch variability. Alternative hydrogels are therefore of interest to advance this model. This new project will investigate the use of alginate gels in our PEA system for the improvement of HSC maintenance and better reliability. Alginate offers a highly tunable and modifiable alternative to collagen that can provide additional benefits to the niche system, offering flexibility in adjusting its properties and has the potential to preserve cells in a more quiescent state.

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Active biomaterials for the *ex-vivo* expansion of Hematopoietic Stem Cells: from biointerfaces to functionalised hydrogels.

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Abstract

Inspired by the huge clinical significance of Hematopoietic Stem Cell (HSC) transplants, we aim to develop a synthetic platform for their expansion, *ex-vivo*. Our system is based on a biointerface between human HSCs, the non-pathogenic bacterial species *Lactococcus lactis* (*L. lactis*), and synthetic polyethylene glycol (PEG) hydrogels, that mimics the chemical and mechanical properties of the bone marrow (BM). The bacteria have been genetically engineered to produce human CXCL12, TPO, VCAM1 and FN, key cytokines for HSC expansion, while the hydrogels can be designed to resemble the BM stiffness and architecture. We can further add a layer of Mesenchymal Stem Cells (MSCs) to more closely mimic the BM microenvironment.

Results suggest that our *L. lactis*-CD34+ cell co-culture system supports HSC phenotype maintenance and can achieve up to 19-fold HSC expansion in 2D experiments. HSC and MSC viability in 3D cultures is not affected by the presence of the hydrogels or the biofilm, while our biointerface can maintain a stem-like phenotype in cultured MSCs for up to 14-days. Finally, we show that the hydrogels can maintain the HSC population in 3D, while their biophysical properties can be specifically tuned to encourage HSC proliferation.

Our data suggests that this novel system has a promising potential to mimic the native conditions that support HSC maintenance and proliferation and induce *ex-vivo* HSC expansion. To our knowledge, this is the first attempt to combine a living biointerface, recombinant signal production, and mechanical support provided by hydrogels for BM and HSC niche engineering.

Graphical abstract

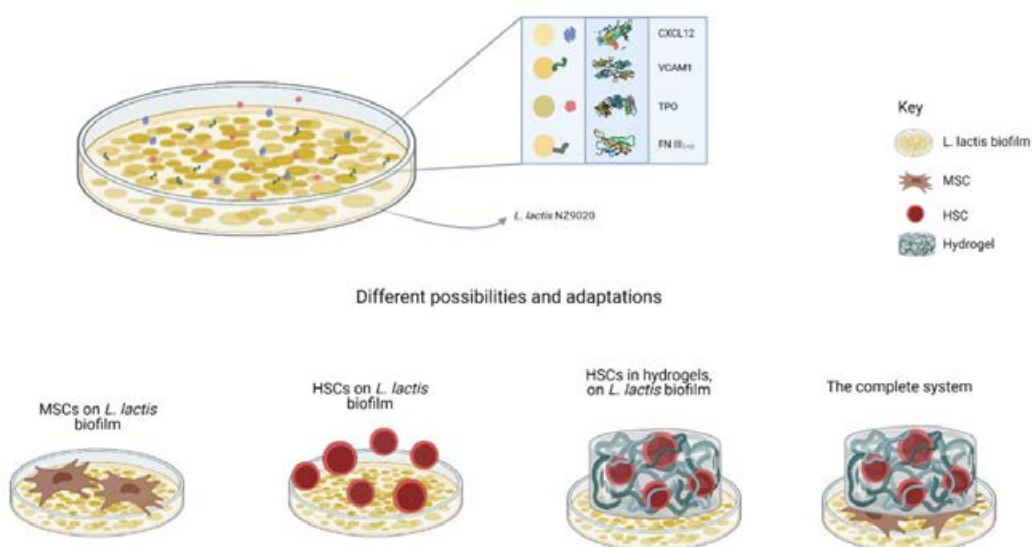


Figure 1. Overview of the system and the various platforms we can create for different applications.

Transgene-free generation of long-term hematopoietic stem cells

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The development of engraftable human long-term hematopoietic stem cells (LT-HSC) from pluripotent stem cells (hPSC) has been a long-sought goal. Definitive HSCs are born from the hemogenic endothelial cells (HEC) residing in the ventral wall of the dorsal aorta (DA) during embryonic development. Blood flow-mediated shear-stress stimulates the endothelial-to-HSC transition (EHT). However, it remains unknown why the ventral wall, and not the dorsal wall, of the DA is the restricted site of the EHT when blood flows through the entire DA, and attempts to mimic blood-flow mediated biomechanical cues have not produced engraftable human HSCs. Since HSCs can form despite the absence of active blood circulation, we analyzed the heartbeat-mediated pulsatile displacement experienced by the zebrafish embryo DA walls. Spinning disc confocal microscopy and single-particle tracking analyses of pulsating blood vessels showed that pulsation-mediated circumferential stretch is restricted to the ventral DA part and responsible for the magnitude, the site, and timing of the HSC formation. We next developed a bioreactor to establish the functional link between blood vessel pulsation and HSC formation. Our serial transplant and limiting dilution assays demonstrated that pulsation-mediated circumferential stretching of HECs activates the mechanoreceptor Piezo1 leading to 3-fold production of mouse LT-HSCs; which reconstitute to normal multi-lineage adult blood. To translate our findings in human setting, we treated human PSC-derived HECs with the Piezo1 activator Yoda1 obtaining the first transgene-free human LT-HSCs that can self-renew, engraft, and reconstitute multi-lineage blood upon serial transplantations, which is critical for generating off-the-shelf transgene-free HSC-based cellular therapies.

Translational control in hematopoietic stem cell maintenance and expansion

Hanna Shin^{*}, Dheeraj Bhavanasi, and Peter S. Klein

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Hematopoietic stem cell transplantation (HSCT) is clinically used for multiple hematopoietic diseases. Umbilical cord-blood (UCB) HSCs require less stringent HLA matching and have reduced risk of graft versus host disease, but most UCB units have insufficient numbers of HSCs for transplant in adults. Ex vivo HSC expansion would greatly improve the utility of UCB for HSCT. However, HSCs lose their capacity for long-term self-renewal in standard culture conditions. Our lab established a culture system that maintains long-term HSCs ex vivo. Using this system, we performed a high-throughput screen (HTS) for hematopoietic stem and progenitor cell (HSPC) expansion and identified multiple compounds that target the translation initiation factor eIF4E and inhibit upstream of eIF4E. As the low rate of translation in HSCs is essential for their function, we selected an eIF4E targeting drug, 4E1RCat, for further analysis. 4E1RCat allowed 10-fold expansion of human HSCs within 4 days as measured by limiting dilution analysis. Therefore, targeting translation initiation may facilitate HSC expansion ex vivo. We will optimize ex vivo HSC expansion using 4E1RCat and test whether 4E1RCat reduces translation in HSCs. And, we will study how eIF4E signaling regulates translation in HSC homeostasis. Our HTS identified multiple compounds that inhibit ERK and p38 MAP kinase pathways, which converge on MNK1 to activate translation through phosphorylation of eIF4E. Thus, we will test whether eIF4E phosphorylation by MNK1 regulates global translation in HSCs and whether inhibition of these pathways facilitates ex vivo expansion of HSCs.

Title: Improved Gene Therapy for metachromatic leukodystrophy

Authors: Lucas Tricoli, Adeline Vanderver, Laura Adang, Max Chappell, Laura Breda, Naoto Tanaka, Michael Triebwasser, Amaliris Gonzalez, Lars Schlotawa, Peter Kurre, Stephanie Hurwitz, Stefano Rivella

Abstract: MLD is an autosomal recessive lysosomal storage disease (LSD) characterized by a mutation of the enzyme Arylsulfatase A (ARSA) most often affecting children in the late infantile stage (LI) (less than 30 months of age at onset), which is the most rapidly progressive form of the disease. Utilizing ex-vivo hematopoietic stem cell (HSC) gene therapy, a functional copy of the ARSA gene replacing mutated ARSA causing MLD, has the potential to restoring proper enzyme activity and has preliminarily demonstrated successful treatment in clinical trials. Current approaches for MLD rely on the use of AAV or lentiviral vectors (LVs), which for AAV may not sustain high level of gene expression in neurological disorders or for LVs requires multiple integrations to prevent the development of the disease. In addition for the currently existing clinical LV these levels are considered insufficient to help symptomatic patients. For all these reasons, we propose that a vector expressing higher levels of ARSA at single integration will reduce the chances of genotoxicity, may be curative in symptomatic patients and, in the future, may also reduce the requirement for full myeloablation. For our top performing vectors, ARSA activity, normalized to VCN, demonstrated that TCO-EAAWP-UTR⁺, TCO-EAFWP-UTR⁻ and TCO-AEAFWP-UTR⁻ showed more ARSA activity (respectively 2X, 10X and 4X) compared to the clinical vector with protein analysis by western blot showing a similar trend. We have also demonstrated with our vectors the ability to secrete more functional ARSA enzyme into the surrounding media in cell culture, which is a critical modality for transfer of functional ARSA from microglia to oligodendrocytes. Current experiments in vector transduced mouse HSC have demonstrated high levels of transduction with superior levels of ARSA activity. Transplanted WT mice are currently living vibrantly with a high level of transduction from our top performing vector.

Fibroblastic stromal cells expressing Delta-like Notch ligands control extrathymic T cell development in mesenteric lymph nodes

Ashley Vanderbeck, Samantha Kelly, Leolene Carrington, Frederick Allen, Joshua Brandstadter, Eric Perkey, Gloria Jih, Anneka Allman, Daniela Gomez-Atria, and Ivan Maillard

Early T cell development is supported by signals mediated by the Notch1 receptor in T lineage progenitors and Delta-like4 (Dll4) ligand in thymic epithelial cells. Although T cell development is restricted to the thymus, extrathymic T cell development has been reported in athymic Foxn1^{nu/nu} nude mice as well as during times of thymopoietic stress such as post-bone marrow transplantation (BMT). A population of CD4+CD8+ double positive T cell progenitors can be found in the mesenteric lymph nodes (MLN) in both athymic mice and early post-BMT, suggesting the presence of an extrathymic niche conducive to T cell development. However, whether Notch signaling is required, as well as the cellular source(s) of Notch ligand throughout this process in the MLN, remains unknown. We hypothesize that MLNs harbor a unique environment in which Notch ligands are available to circulating progenitors and critical to sustain extrathymic T cell development in these contexts. To test this, we utilized systemic neutralizing antibodies as well as loss-of-function genetic models to assess whether the Notch ligands Dll4 or Dll1 are important for extrathymic T cell development. We found that, like in homeostatic thymocyte development, Dll4 appears to play an essential role in generation of early T cell progenitors found in the MLN. Furthermore, the source of Dll4 appears to reside within subsets of non-hematopoietic fibroblastic stromal cells lineage traced by a *Ccl19-Cre* transgene. In sum, these findings shed new light on the cellular and molecular cues regulating T cell development outside of the thymus.

Engineering the pan-leukocyte antigen CD45 to facilitate CAR-T cell therapy against hematologic malignancies

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Abstract

Currently, chimeric antigen receptor (CAR)-T cells must be individually designed for each disease, targeting lineage-associated antigens such as CD19 for B-cell malignancies or BCMA for myeloma. This approach is inefficient and limited to antigens for which on-target/off-tumor toxicities from the CAR-T cells is clinically tolerated. By targeting the pan-hematologic antigen CD45, a single “drug” could be used for most hematologic malignancies, thereby accelerating clinical research and expediting the treatment of more patients. A unique hurdle in developing CD45-directed CAR-T cells is that healthy leukocytes (including CAR-T cells) express CD45 themselves. We hypothesize that engineering the CD45 molecule on hematopoietic cells (including CAR-T cells) to lack the target epitope required for recognition by CAR-45 can generate functional, fratricide-resistant CAR-T cells and a hematopoietic system that is resistant to CD45-targeted CAR-T cell therapy. We show that CD45 CAR-T cells are susceptible to fratricide, as expected. By deleting CD45 in primary human T-cells we prevented fratricide without affecting CAR-T cell function in vitro. Using this approach, we identified three CD45-directed CAR constructs that demonstrate target-specific cytotoxicity against multiple hematologic cancer cell lines (T-ALL, AML and CML). Using truncated versions of the CD45 extracellular domain, we mapped the binding domain that is recognized by the CAR constructs, information that will be used to edit this region using CRISPR/Cas base editing in human hematopoietic stem cells to avoid long-term toxicities.

Transient expansion and myofibroblast conversion of marrow adipogenic lineage precursors (MALPs) mediate bone marrow repair after radiation

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Disclosures: None

INTRODUCTION: Radiotherapy treats malignant tumors effectively but also damages surrounding tissues, such as bone marrow. It is well known that radiation causes a collapse of bone marrow cells and elimination of microvasculature. At a less severe level, radiation damage can be reversed, indicating that bone marrow has a regenerative ability. However, the mechanism governing such recovery is still largely unknown. Apart from hematopoietic cells and endothelial cells, mesenchymal lineage cells are also a major component of bone marrow providing supportive microenvironment for hematopoiesis and angiogenesis. Using single cell RNA-sequencing (scRNA-seq) technique, we recently discovered a novel subpopulation of mesenchymal cells that express most adipogenic markers but with no lipid accumulation and named them marrow adipogenic lineage precursors (MALPs) [1]. In this study, we utilized a modest focal radiation dosage to generate a bone marrow repair model and investigate the underlying mechanism.

METHODS: *Animals*- All animal work was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania. *Col2-Cre Rosa-Tomato (Col2/Td)* mice and *Adipoq-Cre Rosa-Tomato (Adipoq/Td)* mice were generated. For focal radiation, mouse right femur received a clinically relevant radiation dose of 5 Gy using small animal radiation research platform (SARRP). *ScRNA-seq analysis*- Sorted Td⁺ cells from the endosteal bone marrow of 1-month-old *Col2/Td* male mice with no radiation (NR, 2 batches, n=5) or at 3 days after radiation (R, 1 batch, n=3) were subjected to library construction and sequencing. Unsupervised clustering was conducted by UMAP to generate cell clusters of the overall cell populations. Pseudotime trajectory analysis was conducted using Monocle 3. *Whole mount immunofluorescence*- Bones were processed for 50 µm-thick whole mount cryosections and stained with indicated antibodies. *Statistics*- All analyses were conducted using t-tests or one-way ANOVA with Tukey post test.

RESULTS: 5 Gy focal radiation to femurs of 1-month-old mice caused acute damage on bone marrow cellularity and vasculature at day 3, which was recovered at day 14. Using *Col2-Cre* that labels the entire mesenchymal lineage cells in bone [2, 3], we unexpectedly observed a 5.5-fold increase of bone marrow Td⁺ cells at day 3 after radiation, which later gradually disappeared. ScRNA-seq on Td⁺ cells sorted from NR or R bones at day 3 generated the same clustering pattern consisting of early mesenchymal progenitors (EMPs), late mesenchymal progenitors (LMPs), lineage committed progenitors (LCPs), osteoblasts, osteocytes and MALPs and the same pseudotime trajectory pattern (Fig.1 A, B). After integration of 2 datasets, we found that the mesenchymal progenitor pool including EMPs and LMPs was drastically shrunk while MALPs were greatly expanded after radiation (Fig.1 C). Interestingly, cell cycle analysis revealed that MALPs, a non-proliferative cell type, became highly proliferative after radiation (Fig.1.D). Analyzing differentially expressed genes (DEGs) found that radiation upregulates many myofibroblast (*Acta2*, *Tgln*, *Myl9* etc) and extracellular matrix (*Col9a1*, *Col10a1*, *Col1a1* etc) genes in MALPs, indicating a myofibroblast transformation. Using *Adipoq-Cre* to label MALPs *in vivo*, we observed that radiation significantly increased the number of MALPs and its proliferation (EdU⁺Td⁺ cells) at day 3 post radiation (Fig.2). qRT-PCR of sorted Td⁺ cells from *Adipoq/Td* mice confirmed that myofibroblast markers are indeed increased (Fig.3 A). MALP is composed of a cell body with several cell processes. Strikingly, radiation diminished the number of its cell processes and changed its shape to more spindle-like (Fig.4 B). Many MALPs exist as pericytes. Td⁺ pericytes in *Adipoq/Td* mice decreased by 76% along with vessel dilation at day 3 post radiation but returned to normal numbers with relatively normal vessel structure at day 14 (Fig.3 C). Ablation of MALPs in *Adipoq/Td/DTR* mice after radiation almost completely abolished the recovery of bone marrow cellularity (decreased by 85%) and vessel structure at day 14 (Fig.3 D).

DISCUSSION: In the adipogenic differentiation route of bone marrow mesenchymal stem cells, MALPs are situated after mesenchymal progenitors and before lipid-laden adipocytes (LiLAs). They normally do not proliferate. Our study on radiation effects on bone discovered that MALPs start to proliferate and acquire myofibroblastic features after radiation, which are essential for subsequent bone marrow repair. Our data revealed the plasticity of MALPs and shed light on seeking new target for alleviating radiation damage on bone.

SIGNIFICANCE: MALPs play a crucial role in bone marrow regeneration after radiation injury.

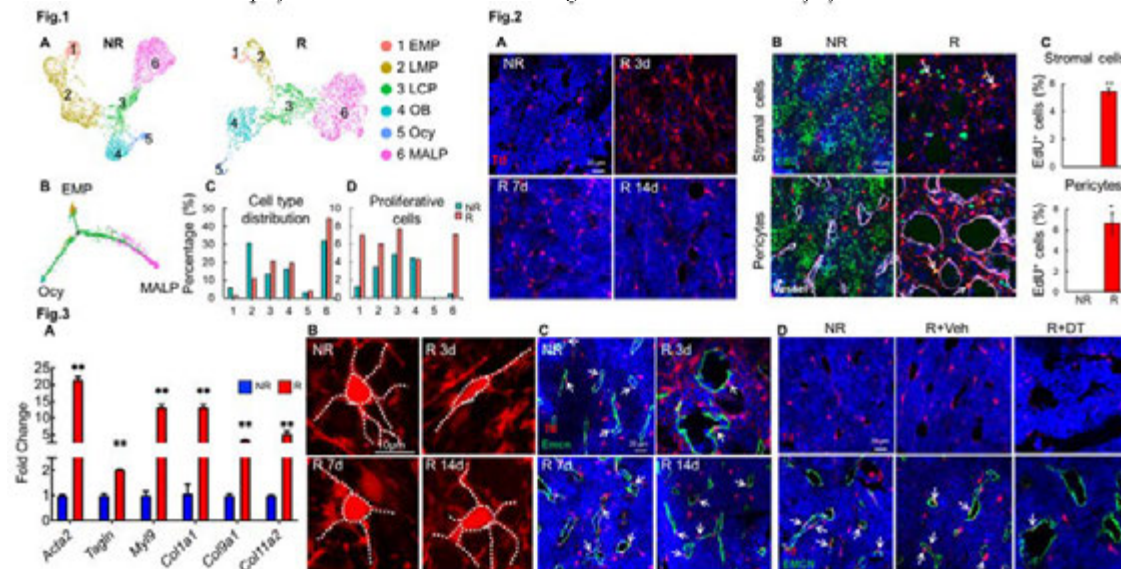


Figure 1. ScRNA-seq analysis predicts MALPs expansion and cell cycle entry after radiation. (A) The UMAP plot of Td⁺ mesenchymal lineage cells isolated from 1-month-old *Col2/Td* mice with no radiation (NR) or at 3 days after radiation (R). (B) Monocle trajectory plot from R dataset. (C) The distribution of cells from NR and R datasets in each cluster.

(D) The percentage of proliferative cells (S/G2/M phase) among each cluster in NR or R conditions was quantified. **Figure 2. Radiation transiently expands MALP pool via stimulating its proliferation.** (A) Fluorescent images of Td⁺ cells in the endosteal bone marrow of 1-month-old *Adipoq/Td* femur before and after focal radiation. (B) Fluorescent images of EdU incorporation in bone marrow cells of *Adipoq/Td* femurs at 3 days post radiation. Arrows point to EdU⁺Td⁺ cells. (C) Quantification of EdU⁺ cells in both stromal and pericytes from *Adipoq/Td* mice. **Figure 3. MALPs acquire myofibroblastic features and are indispensable for bone marrow repair after radiation.** (A) qRT-PCR of sorted bone marrow Td⁺ cells from *Adipoq/Td* mice. (B) Cell processes in 3D image of MALPs. (C) Fluorescent images of vessel and Td⁺ pericytes on vessel post radiation. (D) Fluorescent images of vessel and Td⁺ pericytes on vessel after radiation and DT treatment.

REFERENCES: 1. Zhong, L., et al. *Elife*, 2020. 9: elife.54695. 2. Chandra, A., et al. *J Bone Miner Res*, 2017. 32: jbmr. 2996. 3. Ono, N., et al. *Nat Cell Biol*. 2014.16: ncb3067.

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