Published in final edited form as:

Exp Hematol. 2016 February ; 44(2): 101–105. doi:10.1016/j.exphem.2015.10.006.

Hematopoiesis “awakens”: evolving technologies, the force behind it

Eugenia Flores-Figueroa¹, Marieke Essers², and Teresa V. Bowman^{3,#}

¹Oncology Research Unit, Oncology Hospital, National Medical Center, IMSS, Mexico City, Mexico

²Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM gGmbH), 69120 Heidelberg, Germany; Division of Stem Cells and Cancer, Hematopoietic Stem Cells and Stress Group, Deutsches Krebsforschungszentrum (DKFZ), 69120 Heidelberg, Germany

³Departments of Developmental and Molecular Biology and Medicine (Oncology), Gottesman Institute for Stem Cell Biology and Regenerative Medicine, Albert Einstein College of Medicine, Bronx, NY, USA

Amongst the beauty of the Kyoto countryside, leaders in the field of hematology met at the 44th annual ISEH meeting in late September 2015. Led by ISEH President Paul Frenette and President-Elect David Traver, the meeting covered many aspects of hematopoiesis with a focus on technology. From the meeting, it is clear that the future of hematology is being shaped by innovations in single cell “omics” and imaging approaches that will provide answers to age-old questions on cellular identity. In this meeting review, we highlight the advances presented on understanding the hematopoietic stem cell (HSC) niche, heterogeneity, stress response, epigenetics and how these processes change from birth to old age.

To start off the meeting, Elaine Dzierzak (University of Edinburgh, UK) presented the Donald Metcalf award lecture. She took us on a journey along the most important discoveries in the field of developmental hematology, both by her lab as well as others and at the same time gave us some insights in how her own career, garden and family developed over the years. Dr. Dzierzak’s group was the first to show that HSCs were born in the aorta-gonad-mesonephros (AGM) region of the developing mammalian embryo¹. More recently, through live imaging, they have been able to show that HSCs arise from endothelial cells lining the wall of the embryonic aorta in a process known as the endothelial-to-hematopoietic transition (EHT)². Currently they aim to uncover the molecular program for HSC generation during EHT and define which niche factors can influence this process³.

[#]For correspondence: 1300 Morris Park Avenue, Bronx, NY 10461, teresa.bowman@einstein.yu.edu, phone- (718) 430-4001, fax- (718) 430-8567.

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The stem cell niche: the blind men get closer to “seeing” the elephant

The niche was a major focus of this year’s conference. David Scadden (Harvard University, USA) gave us all a needed historical lesson on the long rode of understanding the stem cell niche. His first slide showed a young Ray Schofield smiling at his office with a picture of “the blind men and an elephant” hanging from his wall⁴; on the following slide he showed a recent picture of Dr. Schofield on a rainy day accompanied by Dr. Scadden on a street in Scotland. These images reminded us that it has been 37 years since Dr. Schofield proposed and created the concept of the stem cell niche⁵. He theorized that the niche would be a limited anatomic site that provides stem cells with the necessary elements to expand, but not exhaust, and to regulate stem cell numbers⁵. Schofield’s concepts on the stem cell niche were validated 22 years later for Germline Stem Cells (GSC) in *Drosophila*, and a few years later in *C. elegans* (reviewed in ⁶). In both models, the niche is a fixed and defined space within the tissue; composed of a limited number of “niche” cells, which are well characterized. But why is it harder to define the HSC niche? Several talks at this year’s meeting provide some insight into the complexity of the HSC niche that might help the “blind men see the elephant” with clearer vision.

Hematopoiesis takes place within the bone marrow, suggesting an interconnection between the skeletal and hematopoietic systems. The bone is constantly remodeling, and both systems are damaged following cancer-related therapies, such as chemotherapy and transplantation. Louise Purton (St. Vincent’s Institute, Australia), presented evidence illustrating several MPP (multi-potent progenitor) niche constituents, including trabecular bone, vessels, osteocytes and adipocytes are altered following chemotherapy⁷. Her work is not only clinically relevant for understanding how to accelerate hematopoietic recovery of cancer patients, but it is also important to consider in the laboratory for comparing results where the conditioning regimens may vary. Yoshio Katayama (Kobe University Hospital, Japan) addressed a clinical observation regarding immunodeficiencies suffered by astronauts⁸. He demonstrated that changes in the bone microenvironment from loss of gravity lead to decreased B cell precursors and abnormalities in the thymic microenvironment due to alterations in both osteoblasts and osteocytes^{9,10}. Dr. Scadden expanded the connections between bone and T-cell production showing depletion of osteocalcin-positive bone cells alters the production of T-biased lymphoid progenitors and T-cells. Ayako Nakamura-Ishizu (National University of Singapore, Singapore) showed the function of megakaryocytes (Mks) as a niche component requires an alternative mechanism of thrombopoietin (TPO) production mediated by CLEC-2 and requiring the interaction of osteoblasts, Mks and HSC¹¹. Takashi Nagasawa (Kyoto University, Japan) presented his work in support of an mesenchymal stem cell (MSC) niche by demonstrating that the transcription factor Foxc1 expressed in CXCL12-abundant reticular (CAR) cells enhances Kit ligand and CXCL12 expression, inhibits adipogenesis, which ultimately alters hematopoietic output¹².

Besides cell populations, the metabolic status and physical conditions, like oxygen tension and permeability, have an impact on both stem cells and niche cells. Hiromitsu Nakauchi (Stanford University, USA) found that the concentrations of various amino acids are higher in bone marrow cells compared to peripheral blood. He further showed that dietary

restriction of one amino acid reduced HSC number and emptied the BM niche. His finding suggests that dietary restriction of this essential amino acid might be a viable alternative to chemotherapy or radiation-based myeloablative regimens for hematopoietic cell transplantation. Regarding niche cells, Peter Carmeliet (Vlaams Instituut voor Biotechnologie, Belgium) and Tsvee Lapidot (Weizman Institute, Israel) explored the heterogeneity within blood vessels in the bone marrow that might contribute to different hematopoietic supportive functions. Peter Carmeliet identified a new role for glycolysis only in angiogenic endothelial cells¹³ and Tsvee Lapidot demonstrated vascular permeability and reactive oxygen species (ROS) are divergent between bone marrow Nestin-positive arteries and Nestin-negative sinusoids, which alters their niche supportive abilities.

Due to the technical challenges of studying the human HSC niche *in situ*, most of the information we have about the niche comes from mouse models with more recent insights from the zebrafish^{14,15}. Human hematopoietic xenotransplantation, first pioneered by John Dick and Dominique Bonnet¹⁶, is an instrumental technique for defining functional human hematopoietic stem and progenitor cells (HSPC), but is limited in its utility due to the lack of a human niche. Many groups have attempted to “humanize” mouse models, improve human xenografts and establish *in vivo* systems to study human HSPC-niche interplay (reviewed in ¹⁷). Markus Manz (Universitäts Spital, Switzerland) reviewed the excellent recent progress along this vein and discussed the use of mouse models expressing human versions of key hematopoietic cytokines, such as Kit Ligand, GM-CSF (Granulocyte-Macrophage Colony-Stimulating Factor), and IL-3 (Interleukin 3), as well as co-transplantation of patient-specific HSC and MSC in myeloma¹⁸ and MDS¹⁹. Satoshi Yoshihara (Hyogo College of Medicine, Japan) cautions the use of the human cytokine models (NSG (NOD-SCID-gamma) mice expressing Kit Ligand, GM-CSF and IL-3 to improve myeloid reconstitution of xenotransplants, as it has been linked to increased myeloid reconstitution with a significant elevation in inflammatory cytokines and hemophagocytic lymphohistiocytosis.

Since 1978, the HSC niche field has made important advancements. We now have a better panoramic view of it, but it is not yet complete. HSC-niche interplay is dynamic and many basic concepts on hematopoiesis are still evolving. The future looks promising with new technologies to track the stem cell niche, but even more important is to maintain and improve the communication between the scientific community (blind men talking to each other), so that we all benefit from the shared perspectives and talk the same talk.

Not all stem cells are equal

Cellular heterogeneity is a long-recognized hallmark of cancer and of cancer stem cells. Current models imply that both cell type and mutational landscape underlie much of tumor heterogeneity²⁰. John Dick (Princess Margaret Cancer Center, Canada), a pioneer of xenotransplantation models to explore the complexity of human HSPC and leukemia-initiating cells, and Irv Weissman (Stanford University, USA), a leader in the field of HSC and progenitor cell purification and identification, presented compelling data about the origins of leukemia initiation. Both Dick and Weissman argued that the precursor to full-blown leukemia is the establishment of clonal dominance within a pre-leukemic stem cell

(LSC)^{21,22}. The pre-LSCs are defined as phenotypically normal, multi-lineage HSCs that carry leukemia-initiating mutations, often in chromatic remodeling genes, which contribute to the cell's clonal expansion through the acquisition of new mutations in pro-proliferative genes. Dick also presented evidence that implied pre-LSCs are involved in relapse. Understanding the identity of these cells and how mutational landscape influences tumor progression are key to unlocking cures for leukemia. For more on clonal evolution of pre-leukemic hematopoietic stem cells in leukemia, check out the ISEH June 2015 webinar featuring Ross Levine and Ravindra Majeti²³.

Cellular heterogeneity is not restricted to tumor biology. The groundbreaking work from Christine Muller-Sieburg and others^{24–26} revealed normal HSCs are also quite heterogeneous (reviewed in ²⁷). Clonal dynamics of HSCs evolve from birth to death with a decrease in HSC function in old animals²⁸. Fernando Camargo (Harvard University, USA) used a novel *in situ* clonal marking system to show that steady-state hematopoiesis in young mice is mostly maintained by long-lived progenitor cells, such as common myeloid progenitors (CMPs) and myeloid-restricted progenitors, with minimal contribution from long-term (LT)-HSCs²⁹. In contrast, in 2-year-old mice, there is an ~4-fold increase in LT-HSC output implying changes in the aging microenvironment could trigger the awakening of these normally dormant cells or diverse HSC populations have different “clocks” dictating the timing when they are active. Isabel Beerman from the Rossi laboratory (Harvard University, USA) presented single-cell fluidigm expression data and DNA methylation analysis and observed a link between DNA methylation changes in LT-HSC and transcriptional alterations in downstream progeny suggesting intrinsic epigenetic changes in old HSCs could modify lineage output. As with life, synergy between nature and nurture likely define what an HSC will be when it grows up.

To get at the roots of cellular heterogeneity, single cell approaches must be employed. Bertie Gottgens (Cambridge University) presented work from his laboratory interrogating the transcriptional heterogeneity of over 4000 single cells. Combining index sorting, fluidigm, and single cell RNA-seq technologies with *in vitro* and *in vivo* validated progenitor and stem cell populations, the Gottgens laboratory has defined the transcriptional wiring that distinguishes functional heterogeneity in murine HSCs³⁰. David Scadden reported single-cell analysis of bone-lining niche cells, which they used to define a new IL-18-expressing niche cell that acts as a regulator of quiescence. The work expands the growing debate on niche cell heterogeneity.

Timm Schroeder (ETH Zurich, Switzerland) has spent years establishing imaging and computational approaches to study single-cell dynamics in real time³¹. At the meeting, he presented data challenging the paradigm of transcription factor competition between PU.1 and GATA1 (reviewed in ³²). Unlike the model, which posits that PU.1 and GATA1 compete in a progenitor cell for directing myeloid or erythroid fate, respectively, Schroeder presented data that GATA1 and PU.1 are rarely expressed in the same cell and whenever PU.1 is present, GATA1 expression is infrequently turned on. These data suggest that an event prior to GATA1 expression in a progenitor directs the bifurcation of myeloid vs. erythroid. To learn more about single-cell approaches in hematopoiesis, check out the ISEH-

sponsored November 2015 webinar on “Comparison of methods for cell labeling and clone tracking” featuring David Kent and Leila Perie.

Beyond the double helix

Epigenetics is a heritable change in gene expression that is not encoded in the DNA sequence, and includes DNA methylation, chromatin modification, and RNA processing. DNA methyltransferases (DNMT), which methylate DNA leading mainly to gene silencing, and TET dioxygenases (TET), which convert methylated DNA to hydroxymethylated DNA leading to mostly gene activation, are thought act in a yin-yang fashion balancing gene expression. Paradoxically, both of these factors are commonly mutated in hematologic diseases, such as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), suggesting imbalance in either direction is detrimental to hematopoiesis³³. Margaret Goodell (Baylor College of Medicine, USA) presented data that TETs limit the function of DNMTs in HSCs at specific regions of the genome, termed canyons, leading to massive expansions of these regulatory elements. Depletion of both DNMT3A and TET2 in HSCs leads to an increase in hematopoietic progenitors and B-cell lymphoma as well as a decrease in red blood cell differentiation. In addition to methylation, chromatin organization can greatly influence gene activation status. Cohesins are molecular glue that function in sister chromatid linkage and gene expression control. Iannis Aifantis (New York University, USA) and Roman Galeev (Larson lab- Lund University, Sweden) showed diminishing cohesion levels, but not complete removal, alters HSC function independent of its effects on cell division³⁴. Aifantis’ group then used ATAC (assay for transposase-accessible chromatin)-Seq to demonstrate that the underlying mechanism is likely through alterations in chromatin confirmation, which then leads to increased expression of erythroid lineage genes and a bias toward the granulocyte-monocyte progenitor stage³⁴.

RNA processing is another major, although less explored, component of epigenetic regulation. Several talks at ISEH focused on the role of RNA binding proteins, showing the broadening interest in this field of gene expression control in hematopoietic research. Daniel Tenen (National University of Singapore, Singapore) previously challenged dogma by showing binding of RNA by the DNA methyltransferase DNMT1 inhibits its methyltransferase activity³⁵. At this year’s meeting, he again challenged the norm, by presenting data that the protein SALL4, which was thought to be a DNA binding transcription factor, actually binds RNA with higher affinity and interacts with many members of the mRNA splicing machinery. SALL4 is overexpressed in MDS and AML³⁶, which are diseases with a high prevalence of mutations in splicing machinery, suggesting the mechanism of SALL4’s role in leukemia could be through splicing regulation. Catriona Jamieson (UCSF, USA) discussed her ongoing studies on the role of RNA editing in leukemic stem cells, demonstrating overexpression of the RNA editase ADAR1 (ADENINE DEAMINASE, RNA-specific-1) leads to altered microRNA processing and leukemia progression³⁷. Takahiro Ito (University of Georgia, USA) focused on another RNA binding protein, MUSASHI-2, and showed that post-transcriptional targeting of Flt3 mRNA by MUSASHI-2 explains part of the leukemogenic potential of MUSASHI-2.

Stressed out stem cells

Another interesting focus of this year's ISEH meeting was the behavior of HSCs under stress conditions, the effect stress has on the function of HSCs and its role in disease development. Utpal Banarjee (UCLA, USA), Toshio Suda (Cancer Science Institute, Singapore) and Amelie Guitart (University of Edinburgh, UK) focused on the role of metabolic pathways, mitochondrial activity and ROS for the function of HSCs, both during development as well as in adult HSCs. Suda for example talked about the role of ASPP1 (apoptosis stimulating protein of p53-1) in leukemia. Methylation of *Aspp1* correlates with poor prognosis in ALL, suggesting it could act as a tumor suppressor in leukemia. *Aspp1*^{-/-} mice have more phenotypic HSCs, perform better in secondary transplants, but also acquire more DNA damage upon irradiation, and in a p53^{-/-} background develop more leukemia.

Several speakers nicely demonstrated the effect of stress on HSC function caused by cancer therapy, hyperactive stress pathways, or transplantation. Purton, as mentioned before, talked about the changes occurring in the microenvironment following different forms of stress, like 5-fluorouracil (5-FU), irradiation, and bone marrow transplantation, that alter the repopulating potential of BM cells⁷. Alan D'Andrea (Dana Farber Cancer Institute, USA) discussed the role of a hyperactive TGF β pathway in Fanconi Anemia (FA) mouse models as well as CD34⁺ FA patient samples. Marieke Essers (DKFZ/HI-STEM, Germany) identified a novel Extracellular matrix protein, Matrilin-4, to play an important role in hematopoietic recovery after 5-FU and the stress of transplantation.

Technology to unite them all

To end the meeting, Andreas Trumpp (DKFZ/HI-STEM, Germany) presented his McCulloch and Till award lecture. He told us about his transformation from a developmental biologist via his work on c-Myc to a stem cell biologist, focusing on the mechanism of dormancy of HSCs and the role of Myc in this process in both HSCs as well as other stem cells like embryonic stem cells (ESCs). Trumpp described the amazing scientists who he worked with through his journey starting in Heidelberg, then California and Lausanne, and then finally a return to Heidelberg as the head of HI-STEM. As a fitting finale of this tech heavy meeting, Trumpp presented what he has learned from developing and employing many new "omics" approaches to examine gene expression, proteomes, and epigenetic regulation of HSC populations as well as single cells³⁸. We look forward to seeing how our perception of HSC biology evolves with these new viewpoints.

Concluding Remarks

Novel technologies were a major force behind many of the new discoveries presented at this year's ISEH meeting. Advances in clonal tracking, genome sequencing and imaging analysis have pushed our field into new arenas and forced us to challenge our previous notions on topics as diverse as pre-leukemic stem cells, clonal dynamics, aging, niche and epigenetic control. It is an exciting time to be in hematology research. May the "force awaken" new ideas in us all.

Acknowledgments

This work was funded by CONACYT (grant no. CB-2012-01-179417), Fondo de Investigación en Salud (grant no. FIS/IMSS/PROT/21) and the Programa de Cooperación Internacional- IMSS (EF-F) and Gabrielle's Angel Foundation Scholar Grant and 1R03DK102975-01 (TVB).

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