© 2015. Published by The Company of Biologists Ltd | Development (2015) 142, 1728-1732 doi:10.1242/dev.121103

MEETING REVIEW

Hematopoietic development at high altitude: blood stem cells put to the test

Ann C. Zovein[1] and E. Camilla Forsberg[2,*]

ABSTRACT

In February 2015, over 200 scientists gathered for the Keystone Hematopoiesis meeting, which was held at the scenic Keystone Resort in Keystone, Colorado, USA. The meeting organizers, Patricia Ernst, Hanna Mikkola and Timm Schroeder, put together an exciting program, during which field leaders and new investigators presented discoveries that spanned developmental and adult hematopoiesis within both physiologic and pathologic contexts. Collectively, the program highlighted the increasing pace of new discoveries and the substantial progress made in the hematopoiesis field since the last Keystone meeting two years ago. In this Meeting Review, we highlight the main concepts discussed at the conference, with an emphasis on topics relevant to developmental biology.

KEY WORDS: Hematopoiesis, Pathologic, Physiologic

Introduction

The 2015 Keystone Hematopoiesis Symposium brought together an exciting mix of basic and clinical researchers whose interests covered a spectrum of topics, including stem and progenitor cell development, the maintenance and differentiation of blood lineages during homeostasis, and the impact of stress and disease on the hematopoietic system. Presentations detailed the regulation of these processes by signaling pathways, epigenetic mechanisms, transcriptional and miRNA networks, cell metabolism, and local and systemic factors. Experts at various career stages and from numerous institutes across several countries and continents participated. The meeting had a distinctly international flavor, with a diversity of approaches, model organisms and opinions leading to stimulating, open-minded and fruitful discussion. Scientists eagerly challenged both old dogma and newly emerging concepts. A recurrent theme that was echoed in several presentations was the amazing ability of the hematopoietic system to adapt cellular output in response to stress and a dynamic demand for different mature cell types. Indeed, deep discussions on this topic carried over from the formal scientific sessions to the self-experimentation performed in real time as scientists pondered the effects of high altitude on their own hematopoietic output during skiing, snowshoeing and dancing.

Here, we summarize the meeting, focusing on the topics that we believe are of greatest interest to the readers of *Development*.

Understanding hematopoietic development *in vivo*

Understanding the emergence and progressive maturation of the first hematopoietic stem cells (HSCs) or hematopoietic stem/progenitor cells (HSPCs) *in vivo* can serve as a paradigm for generating these cells *ex vivo*. The initial formation of long-term HSCs occurs within embryonic vascular sites from specialized precursor cells collectively termed hemogenic endothelium (HE). How these precursors are specified and how their conversion to hematopoietic cells is regulated are areas of great interest to the hematopoiesis community and to researchers interested in regenerative therapies. The discussion of developmental hematopoiesis in vertebrates encompassed signaling pathways involved in hematopoietic emergence and expansion, the regulation of hematopoietic fate as hematopoietic cells bud from endothelium and the migration of hematopoietic cells from the endothelium to subsequent niches in the embryo.

Inflammation has recently been implicated as an integral factor during HSC emergence from HE (Espín-Palazón et al., 2014; He et al., 2015; Li et al., 2014; Sawamiphak et al., 2014). In line with this, David Traver (University of California San Diego, USA) shared his work evaluating the role of tumor necrosis factor (TNF) in HSC emergence from the endothelium (Espín-Palazón et al., 2014). Using zebrafish morphants, he demonstrated that, whereas loss of TNFα and its receptor had no apparent effects on vasculogenesis, the loss of inflammatory signaling resulted in significantly decreased hematopoiesis. The TNF receptor TNFr2 is thought to regulate Jag1 signaling in aortic endothelial cells, which in turn regulates Notch activity in HE for the hematopoietic fate switch. Thus, inflammatory pathways work upstream of Notch regulation during HSC emergence. Interestingly, vitamin D pathways, which are thought of as being anti-inflammatory, also play a crucial role. Trista North (Harvard Medical School, Boston, USA) presented work on how vitamin D-related compounds modulate the formation of HSCs from HE. She noted that deficient vitamin D receptor activity severely reduced the number of hematopoietic cells in the zebrafish embryo, whereas treatment with active vitamin D increased hematopoietic production during all stages of HSC emergence. Functionally, vitamin D supplementation resulted in significantly improved auto-recovery after irradiation, suggesting that it is an important pharmacological modality for expanding HSCs *ex vivo*. Along similar lines of understanding developmental signaling and translating this to *ex vivo* expansion of HSCs, Len Zon (Harvard Medical School, Boston, USA) presented work characterizing the role of prostaglandin E2 (PGE2) in hematopoietic regulation. Earlier work from a chemical screen identified PGE2 as a key regulator of embryonic HSC emergence (North et al., 2007). To further understand the role of PGE2 during hematopoietic development, Zon and colleagues treated zebrafish embryos with PGE2 during development and noted increased numbers of hematopoietic cells in the adult zebrafish kidney. In addition, PGE2 enhanced long-term reconstitution by increasing the expression of cell-cycle regulators in short-term HSCs (ST-HSCs), without affecting long-term HSCs (LT-HSCs). Zon also revealed adenosine as a novel regulator of hematopoietic development. The adenosine receptor was shown to be present on HE; however,

[1]Cardiovascular Research Institute and Department of Pediatrics, Division of Neonatology, University of California San Francisco School of Medicine, San Francisco, CA 94143, USA. [2]Institute for the Biology of Stem Cells, Department of Biomolecular Engineering, University of California Santa Cruz, Santa Cruz, CA 95064, USA.

*Author for correspondence (cforsber@ucsc.edu)

Development (2015) 142, 1728-1732 doi:10.1242/dev.121103

adenosine morphants formed hematopoietic cells from HE that then burst after budding. The failure of cells to survive after budding suggests that adenosine is required after the initial endothelial-to-hematopoietic fate switch. Following on from this, Ann Zovein (University of California San Francisco, USA) presented work investigating the changes that occur prior to this hematopoietic budding stage. During the acquisition of hematopoietic morphology, the transcription factors Sox17 and Runx1 appear to serve opposing roles. It was noted that Sox17 is downregulated as cells acquire a hematopoietic fate, and that it might serve to repress Runx1 transcriptional expression and hematopoietic fate transition. Collectively, these findings implicate novel pathways in hematopoietic specification and offer new means of understanding the earliest stages of this process.

Once hematopoietic fate is acquired from HE and cells bud from the endothelium, multiple steps are involved as these cells transition towards maturation. This was beautifully demonstrated during Zon's presentation, in which he played an animated film depicting the 'birth' of a blood cell from the zebrafish endothelium and its subsequent migratory course. After cells detach from the zebrafish dorsal aorta, they migrate via a non-circulatory route towards the caudal hematopoietic tissue (CHT). An endothelial cell of the CHT vasculature then envelops the entering HSC. The HSC then undergoes an oriented cell division, and one daughter cell immediately enters the circulation, while the other remains in the CHT niche (Tamplin et al., 2015). The chemokine CXCL12 is required in CHT niche cells for this interaction, and its receptor CXCR4 is expressed on HSCs. Time-lapse imaging from the Zon group implicated a new cell player – the macrophage – in the release of budding HSCs from the aorta to allow for egress into the CHT. If macrophage populations are depleted in the zebrafish, HSCs appear to remain 'stuck' to the aorta, probably due to overabundant matrix interactions that the macrophages help degrade. This hypothesis fits well with data presented by Trista North, whereby primitive myeloid cells provide signaling required for HSCs in a network in which macrophages precede neutrophils. In this context, the inhibition of Mmp2/Mmp9 activity also reduced HSC emergence. These data suggest that other hematopoietic cells in the embryo play non-cell-autonomous roles in HSC emergence, and that inflammatory mechanisms are probably at play. Continuing the theme of non-cell-autonomous influences, Wilson Clements (St. Jude's Children's Research Hospital, Memphis, USA) investigated the role of the niche in HE. His work demonstrated that the sclerotome compartment of the somite, without directly contributing to the endothelium or blood, is required during HSC emergence during zebrafish embryogenesis. This work suggests an origin for the niche cells that regulate HSC emergence and, consequently, the later steps of hematopoiesis.

Recapitulating hematopoietic development *in vitro*

Understanding and recapitulating the development of HSCs *in vitro* remains a significant challenge in the field, one that has important therapeutic implications. Gordon Keller (McEwen Centre for Regenerative Medicine, Toronto, Canada) discussed how lessons learned from the embryo can be instructive in generating HSCs from embryonic stem cells (ESCs), but also pointed out that the direct accessibility of ESC models for manipulation will lead to new insights into embryonic development. Multiple additional talks focused on developing HSCs *in vitro*, via the differentiation of ESCs, the direct reprogramming of fibroblasts and the differentiation of induced pluripotent stem cells (iPSCs).

Work presented by Keller, as well as by his postdoctoral fellow Andrea Ditadi, showed that multiple types of endothelium can be generated as ESCs differentiate along mesodermal lineages, including cells with arterial and venous signatures. Interestingly, they defined an endothelial subset with hemogenic capacity that did not have arterial or venous signatures, suggesting that HE cells are distinct from their non-hemogenic endothelial counterparts. Based on surface marker expression, they were able to isolate HE precursors from day-8 embryoid bodies (EBs), and they identified an HE subset that produced hematopoietic cells in functional assays, whereas arterial and venous subsets produced only vascular cells in culture. Kateri Moore (Icahn School of Medicine at Mount Sinai, New York, USA) presented her work on generating HSCs from mouse and human fibroblasts. Of the four crucial transcription factors required for reprogramming fibroblasts to a hematopoietic fate (Pereira et al., 2013), she noted that Gfi1-b, cFos and Gata2 were necessary, whereas Etv6 enhanced the process. Translating these findings to the human system, she found that GFi1-b, cFos and Gata2 reprogrammed human fibroblasts into HSPCs that repopulated primary immunocompromised recipients for 12 weeks but did not engraft upon secondary transplantation. In the mouse studies, she and her co-workers noted HE precursors of a Prominin1^{+}/Sca1^{+}/CD34^{+}/CD45^{-} phenotype, and next tested whether this phenotype was present in hemogenic cells *in vivo*. They first analyzed the placenta and indeed found this population, which, once isolated and cultured, further could produce the whole repertoire of *in vitro* clonogenic cells. When transplanted into immunocompromised mice, these cells repopulated as T and B cells, and bone marrow populations isolated from the transplanted mice could repopulate the T and B cell compartment in secondary recipients. The hemogenic precursors were also present in the aorta-gonad-mesonephros (AGM). In conclusion, this was the first demonstration of how direct reprogramming studies can further our understanding of normal developmental hematopoiesis. Another strategy to produce HSCs *in vitro* includes the differentiation of human iPSCs. Sergei Doulatov, a postdoctoral fellow from George Daley's laboratory (Howard Hughes Medical Institute/Harvard Medical School, Boston, USA), evaluated tailored combinations of factors that could produce CD34^{+}/CD45^{+} HSPCs from human iPSCs, including HoxA9, Erg, RORA, Sox4 and myb (Doulatov et al., 2013). They then rescreened for genes imparting lymphoid potential and found 13 transcription factors that were sufficient to produce T and B lymphoid cells. In addition, selective transcription factor modulation was able to impart a lymphoid or myeloid bias to the newly generated HSPCs. Collectively, these presentations gave exciting new insights into the developmental origins of HSCs and the potential to generate HE using reprogramming techniques.

Developmental hematopoiesis requires not only the emergence of HSCs, but also their subsequent migration and maturation within the embryo. Conference participants suggested that the limited engraftment capabilities of *de novo*-generated HSCs are due to a requirement of a fetal liver niche for maturation into functional HSCs. To this end, Hanna Mikkola (University of California Los Angeles, USA) analyzed the differentiation block in generating engraftable HSCs from EBs. By evaluating populations of cells from human placenta, fetal liver and EBs, she identified signaling pathways upstream of Hox genes that might be responsible for the maturation process, highlighting the fact that developmental processes that occur in the embryo are important to consider when recapitulating hematopoietic development *in vitro*.

Development (2015) 142, 1728-1732 doi:10.1242/dev.121103

New perspectives on old players

Another inspiring conference theme was the uncovering of new and unexpected roles of well-known 'players' in hematopoiesis. Andreas Trumpp (German Cancer Research Center, Heidelberg, Germany) and Iannis Aifantis (New York University, USA), for example, presented novel findings on myc, a well-known transcriptional regulator of hematopoiesis. Trumpp's studies revealed that quiescent, or 'dormant', mouse HSCs have very low levels of c- and N-myc, which are increased upon exit from quiescence. The profiling of histone modifications led to the identification of a blood-specific c-myc enhancer, the deletion of which caused a block in HSC differentiation and accumulation of early progenitors without affecting HSC survival. Likewise, genetic deletion of both *c-myc* and *N-myc* was detrimental for hematopoiesis but did not affect the numbers of dormant HSCs. Thus, the survival of dormant HSCs is independent of overall myc activity, whereas combined c- and N-myc activity is essential for the proliferation, survival and differentiation of all other hematopoietic stem/progenitor and mature cells. Similarly, Aifantis found that the ubiquitin-mediated degradation of c- and N-myc is an essential mechanism for limiting myc-mediated proliferation in the context of myelodysplastic syndrome (MDS). He also demonstrated that the E3 ligase Fbxw7 targets both c-myc and N-myc, whereas HUWE1 only ubiquitylates N-myc. Thus, Aifantis proposed that leukemia driven by myc benefits from use of inhibitors of bromodomain and extraterminal (BET) family proteins, which inhibit myc-dependent chromatin modification and gene activation.

Michael Rieger (Goethe University, Frankfurt, Germany) investigated STAT5-dependent regulation of hematopoiesis. Rieger's group found that HSC self-renewal in response to thrombopoietin (Tpo) led to STAT5-mediated upregulation of microRNA-193b (miR193b), which was shown to directly target c-kit expression. Deletion of miR193b led to expansion of functional HSCs in mice, whereas ectopic miR193b expression restricted HSC expansion and hematopoietic reconstitution. Conversely, the absence of miR193b caused an increased STAT5 and Akt signaling response in HSCs. Continuous live-cell tracking experiments *in vitro*, using technology developed by Timm Schroeder (ETH Zürich, Basel, Switzerland), revealed that the mechanism of HSC expansion involved slowed HSC differentiation and a shift towards self-renewing divisions. Thus, Tpo-initiated signaling leads to a STAT5/miR193b/c-kit feedback-loop that regulates the balance of HSC self-renewal and differentiation by controlling cytokine signaling.

A new perspective on how angiopoietin 1 (Ang1) regulates HSC function was also provided. Whereas previous data indicated that osteoblast-expressed Ang1 influences HSC function, Sean Morrison (University of Texas Southwestern, Dallas, USA) reported that HSCs themselves express the highest levels of Ang1. HSCs also express the Ang1 receptor, Tek/Tie2, raising the possibility of autocrine Ang1 signaling. However, the cell-type-specific deletion of Ang1 had no effect on either HSC maintenance or quiescence. Instead, Morrison found that Ang1 acts to regulate niche recovery after irradiation damage. From a developmental perspective, Zon's group demonstrated that angiopoietins are required for HSPC emergence and HE specification, and that they might do so by regulating Notch signaling (Lin et al., 2015). Another twist of events was presented by Patricia Ernst (University of Denver, USA), who reported the surprising discovery that the histone methyltransferase Mll1, a well-known player in hematopoiesis and leukemia, does not depend on its enzymatic activity to regulate HSC function or leukemogenesis. Instead, she showed that Mll1 regulates transcription via recruitment of MOF, a histone acetyltransferase. MOF appears to counteract the deacetylase activity of SIRT1, as SIRT1 inhibition was sufficient to rescue the aberrant acetylation patterns observed upon deletion of Mll1. Nancy Speck (University of Pennsylvania, Philadelphia, USA) presented her latest work investigating Runx1 (AML1), which she first described for its crucial role in definitive hematopoiesis and in leukemic transformation. By evaluating Runx1 mutants via ribosome profiling, she uncovered a provocative role for Runx1 in ribosome biogenesis. The connection between Runx1 and the translational machinery of the cell suggests that Runx1 coordinates the downstream protein synthesis required for HSC self-renewal and clonal expansion of progenitors and/or leukemic cells.

Stress responses and the development of leukemia

In addition to playing an important role in hematopoietic specification, inflammation and other stress situations have tremendous effects on hematopoietic homeostasis and the development of cancer. In line with this, Markus Manz (University of Zurich, Switzerland) demonstrated that toll-like receptor 4 (TLR4) can mediate lipopolysaccharide (LPS)-induced hematopoietic responses by both direct and indirect mechanisms. He reported that LPS-induced emergency granulopoiesis does not depend on hematopoietic TLR4 expression, but that LPS can also enhance myeloid differentiation by directly activating TLR4 expression in HSPCs. Manz noted that, whereas the emergency TLR4 response is beneficial for fighting infectious disease, chronic immune stimulation might lead to inflammation-associated malignant transformation. Emmanuelle Passegué (University of California San Francisco, USA) showed that emergency myelopoiesis is mediated by the clonal expansion of granulocyte macrophage progenitors (GMPs). Imaging of the bone marrow during myeloid regeneration revealed transient clusters of GMPs, which resolve during normal hematopoietic differentiation but persist in myeloproliferative disease as the result of sustained production. The role of inflammation as a double-edged sword for HSC biology was further demonstrated by Eric Pietras, a postdoctoral fellow in the Passegué laboratory (University of California San Francisco, USA), who showed that the pro-inflammatory cytokine interleukin-1 (IL-1) directly increases expression of the transcription factor PU.1 and accelerates myeloid differentiation by HSCs. Whereas such a mechanism is appropriate for driving short-term hematopoietic recovery from insults such as 5-fluorouracil, Pietras showed that chronic IL-1 exposure *in vivo* remodels the hematopoietic system in a way that restricts HSC lineage output and ultimately impairs the ability of HSCs to self-renew. Like myelopoiesis, megakaryopoiesis can rapidly respond to emergency situations. Marieke Essers (Heidelberg Institute for Stem Cell Technology, Germany) described her discovery of a population of stem cell-like megakaryocyte progenitors, disguised phenotypically as HSCs, that functions as an emergency reservoir for platelet generation in response to inflammatory signals. This population might overlap with the von Willebrand factor (Vwf)-expressing cells described by Claus Nerlov (University of Oxford, UK), which displayed a strong platelet bias in transplantation assays. Together, this work revealed novel molecular and cellular regulators mediating the tremendous capability of the hematopoietic system to respond to signals from their surroundings.

Like Essers and Nerlov, Connie Eaves (Terry Fox Laboratory and University of British Columbia, Vancouver, Canada) emphasized that the HSC compartment is functionally heterogeneous; elegant experiments from her group, using mouse and human cells, single-cell transplantation, barcoding and 40-parameter flow cytometry (CyTOF) data, have demonstrated that HSCs are intrinsically heterogeneous at the molecular level. Similarly, Camilla Forsberg

Development (2015) 142, 1728-1732 doi:10.1242/dev.121103

(University of California Santa Cruz, USA) used a lineage-tracing model to show that the fetal liver compartment contains at least two distinct populations of HSCs that, as demonstrated by transplantation experiments, possess intrinsic differences in differentiation potential. An additional level of complexity imparted by extrinsic regulators was conveyed by Eaves' 'pluralistic model' of hematopoiesis, a concept that integrates cell-intrinsic heterogeneity with the multitude of signals provided by external cues. Clonal analysis was also advocated by the keynote speaker John Dick (Princess Margaret Cancer Center, Toronto, Canada). Dick presented new insights into how expansion of subsets of cells can lead to clonal dominance as well as the evolution of disease characteristics and therapeutic response during cancer progression. Investigating the relationship between leukemic and pre-leukemic cells, Dick emphasized the importance of cell-cycle regulation, exemplified by CDK6 expression, in governing HSC clonal expansion, and he discussed how this, together with perturbations such as *Flk2/Flt3* mutations, can precipitate cancer. High-resolution sequencing analysis indicated that cancer relapse can also be of multiclonal origin, initiated by leukemic stem cells (LSCs) that existed at the time of diagnosis, as well as pre-leukemic LSCs that evolved during disease progression. Dick also showed that pre-leukemic clones, often marked by mutations in the gene encoding the DNA methyltransferase Dnmt3a, tend to survive during remission, and he cautioned that measures of therapeutic success should include screening for the existence of pre-leukemic clones in addition to leukemic cells. The importance of Dnmt3a in protecting HSCs from transformation was echoed by Margaret Goodell (Baylor College of Medicine, Houston, USA), who showed that Dnmt3a influences myeloid versus lymphoid fate in both normal and leukemic hematopoiesis. Her model of Dnmt3a as a crucial regulator of the stem cell epigenome, with mutations or genetic deletion leading to clonal dominance over time, particularly in combination with activating mutations in *Flk2/Flt3*, fit well with the ideas conveyed by Dick. Altogether, the speakers painted an increasingly complex picture of leukemic transformation. Nonetheless, hope for a universal strategy for cancer treatment was conveyed by Irving Weissman (Stanford University, USA), who presented studies using anti-CD47 antibodies to eliminate a wide variety of cancer cells. Although Weissman was optimistic about the strategy of using CD47 as a single therapeutic, he suggested that combining this approach with existing therapies, such as Rituximab (a monoclonal antibody that targets CD20), is the most efficient way to eradicate cancer cells.

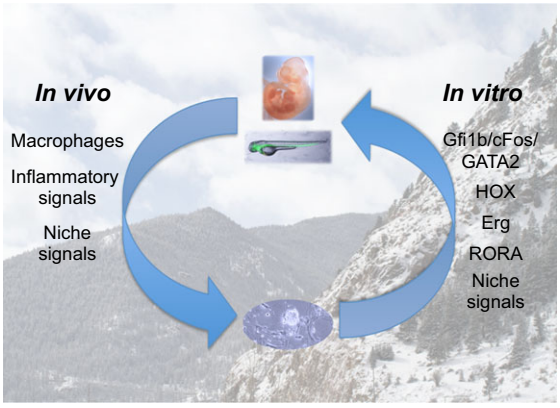

Fig. 1. Developmental themes at the 2015 Keystone Hematopoiesis Symposium. New regulators of developmental hematopoiesis that were reported include intrinsic and extrinsic inflammatory pathways, as well as extrinsic contributions of macrophage populations and niche components. An understanding of these pathways, studied *in vivo* (left arrow), can aid the derivation of HSCs *in vitro* (right arrow). Differentiation to a hematopoietic fate *in vitro* requires multiple developmental regulators that include HoxA9, Erg and RORA in human iPSCs; Gfi1b, cFos and GATA2 in the directed differentiation of fibroblasts; and niche signals from fetal liver and placental populations. Populations identified during *in vitro* differentiation are noted to be also present in the developing organism, thus aiding our understanding and analysis of *in vivo* hematopoiesis.

New and improved technology raises the bar

One of the goals of the meeting was to provide insights into how the integration of innovative tools, such as single-cell quantification and novel imaging technologies, in different model systems has led to new discoveries. It was abundantly clear that the development of robust and sensitive technologies continues to improve the ability to interrogate biological systems with greater sensitivity and at higher resolution. Timm Schroeder presented new insights into cell fate, using continuous tracking of live cells *in vitro* – a system that he has been developing over the past several years and that was also used by Rieger and Pietras. Impressive technologies to track the fate of HSCs *in vivo* were reported and implemented by several other presenters. Gerald de Haan (University Medical Center Groningen, Netherlands) and Eaves both described the use of lentiviral barcoding to track the contribution of mouse and human HSCs to hematopoietic reconstitution under a variety of conditions. Jianglong Sun, of the Camargo lab (Harvard University, Boston, USA), presented data from an *in vivo* cell-tracking approach based on the sleeping beauty transposon system (Sun et al., 2014). Furthermore, Paul Frenette (Albert Einstein College of Medicine, Bronx, USA) and Morrison revealed how they have pushed the limits of bone-marrow imaging technologies to new levels as they continue to define the relevant cellular components, architecture and functions of the HSC niche. Although it will require substantial technological advancement before we reach a comprehensive understanding of HSC trafficking in mammals relative to that in the more tractable zebrafish conveyed in Zon's movies, the ability to localize individual HSCs within the three-dimensional context of mouse bone marrow instills hope that new insights into HSC regulation by the niche will soon be realized.

Conclusions

It was a shared sentiment that these are exciting times to be studying hematopoiesis. We are continuing to derive exciting new insights into cell behavior and gene regulation from blood. During the meeting, new pathways in developmental hematopoiesis emerged that will be instructive during the recapitulation of HSC development in culture. In turn, successful strategies to generate HSCs in culture are providing new insights into embryonic development. Novel roles for well-studied pathways are being discovered, and the biology of HSC emergence and differentiation is becoming more complex and nuanced than ever before. The advent of new technologies and a willingness to challenge dogma is fundamentally changing our views of hematopoiesis. Major challenges remain in synthesizing these findings into a comprehensive understanding of the hematopoietic system. Currently, our simplified models of hematopoiesis fall far short of accurately conveying the regulation of HSC function. Even relatively simple and well-studied aspects of HSC function were subject to debate, and the highly dynamic nature of the hematopoietic system during regeneration or responses to inflammation was reflected in many presentations. In the spirit of Confucius, we agree that "real knowledge is to know the extent of one's ignorance." Thus, while the field continues to gather high-resolution data at a rapid pace, it is also necessary to pause and reflect on the meaning of these findings, and

Development (2015) 142, 1728-1732 doi:10.1242/dev.121103

find new ways to address their current limitations. However, the abundant willingness to engage in open and constructive debates displayed at the meeting instills hope that the field is on track for a deeper and ever-increasing comprehension of the hematopoietic system.

Acknowledgements

The authors thank all meeting participants for engaging in stimulating scientific exchange. Many important findings had to be left out of this Review due to space constraints. We thank the speakers whose work is mentioned for their willingness to share their unpublished work at the meeting and allowing us to summarize their findings here, and for their timely review of the material. We also gratefully acknowledge Wilson Clements and Eric Pietras for their insightful comments on the manuscript. We thank Keystone Symposia and the meeting organizers for their hard work and for successfully implementing an exciting and interactive program.

Competing interests

The authors declare no competing or financial interests.

Funding

We acknowledge the funding support for the authors: the California Institute for Regenerative Medicine [NF3-06479] to A.C.Z.; and awards from the American Cancer Society [Research Scholar Award RSG-13-193-01-DDC], the University of California Cancer Research Coordinated Committee, National Institutes of Health/National Institute of Allergy and Infectious Diseases (NIH/NIAID) [R21AI103656], NIH/National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) [R01DK100917], NIH/National Heart, Lung and Blood Institute (NHLBI) [R01HL115158] and Alex's Lemonade Stand Foundation to E.C.F. Deposited in PMC for release after 12 months.

References

Doulatov, S., Vo, L. T., Chou, S. S., Kim, P. G., Arora, N., Li, H., Hadland, B. K., Bernstein, I. D., Collins, J. J., Zon, L. I. et al. (2013). Induction of multipotential hematopoietic progenitors from human pluripotent stem cells via respecification of lineage-restricted precursors. *Cell Stem Cell* **13**, 459-470.

Espín-Palazón, R., Stachura, D. L., Campbell, C. A., García-Moreno, D., Del Cid, N., Kim, A. D., Candel, S., Meseguer, J., Mulero, V. and Traver, D. (2014). Proinflammatory signaling regulates hematopoietic stem cell emergence. *Cell* **159**, 1070-1085.

He, Q., Zhang, C., Wang, L., Zhang, P., Ma, D., Lv, J. and Liu, F. (2015). Inflammatory signaling regulates hematopoietic stem and progenitor cell emergence in vertebrates. *Blood* **125**, 1098-1106.

Li, Y., Esain, V., Teng, L., Xu, J., Kwan, W., Frost, I. M., Yzaguirre, A. D., Cai, X., Cortes, M., Maijenburg, M. W. et al. (2014). Inflammatory signaling regulates embryonic hematopoietic stem and progenitor cell production. *Genes Dev.* **28**, 2597-2612.

Lin, M. I., Price, E. N., Boatman, S., Hagedorn, E. J., Trompouki, E., Satishchandran, S., Carspecken, C. W., Uong, A., DiBiase, A., Yang, S. et al. (2015). Angiopoietin-like proteins stimulate HSPC development through interaction with Notch receptor signaling. *Elife* **4**, e05544.

North, T. E., Goessling, W., Walkley, C. R., Lengerke, C., Kopani, K. R., Lord, A. M., Weber, G. J., Bowman, T. V., Jang, I.-H., Grosser, T. et al. (2007). Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature* **447**, 1007-1011.

Pereira, C.-F., Chang, B., Qiu, J., Niu, X., Papatsenko, D., Hendry, C. E., Clark, N. R., Nomura-Kitabayashi, A., Kovacic, J. C., Ma'ayan, A. et al. (2013). Induction of a hemogenic program in mouse fibroblasts. *Cell Stem Cell* **13**, 205-218.

Sawamiphak, S., Kontarakis, Z. and Stainier, D. Y. R. (2014). Interferon gamma signaling positively regulates hematopoietic stem cell emergence. *Dev. Cell* **31**, 640-653.

Sun, J., Ramos, A., Chapman, B., Johnnidis, J. B., Le, L., Ho, Y.-J., Klein, A., Hofmann, O. and Camargo, F. D. (2014). Clonal dynamics of native haematopoiesis. *Nature* **514**, 322-327.

Tamplin, O. J., Durand, E. M., Carr, L. A., Childs, S. J., Hagedorn, E. J., Li, P., Yzaguirre, A. D., Speck, N. A. and Zon, L. I. (2015). Hematopoietic stem cell arrival triggers dynamic remodeling of the perivascular niche. *Cell* **160**, 241-252.