Blood stem cells: from beginning to end

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ABSTRACT

In June 2016, around 200 scientist from all over the world gathered at EMBL headquarters in Heidelberg, Germany to discuss the recent advances in hematopoietic stem cells from three different angles: developmental, adulthood and aging. The meeting, aptly named "Hematopoiesis: from the embryo to the aging organism" also covered cutting-edge technologies applied to this subject such as single cell analysis, reprogramming and imaging. This meeting review summarizes the exciting work that was presented and covers the main themes that emerged from the meeting.

KEYWORDS

Hematopoietic Stem Cells, aging, development, single cell genomics, cell fate, imaging technologies.

Introduction

For two full days in early June 2016, the EMBL campus at Heidelberg, a scientific hub in the heart of Europe, hosted hematopoietic stem cell (HSC) specialists from all over the world. For this exciting conference, a new generation of HSC research scientists, Karima Kissa, Christopher Lancrin, Cristina LoCelso and Catherine Robin brought together leading scientist with a common interest in HSC Biology. The purpose of the meeting was to discuss the recent advances in understanding how HSC biology changes through time: from the early decisions that define HSC emergence in the embryo to the effects of aging on HSC maintenance and integrity. The program of the meeting was carefully shaped to cover key aspects of HSC generation, homeostasis, aging and disease. A special emphasis was put on new technologies such as single cell genomics and advanced imaging techniques. It was the use and development of these new technologies to answer old questions that brought the most exciting discussions and new ideas to the meeting. In this Meeting review, we will summarise the main themes that emerged from the various presentations and discussions, and consider where this field is headed in the years to come.

Hematopoietic stem cells in the embryo

The meeting started with a full session on embryonic blood development in different animal models, which highlighted the advantages of using each single model. Zebrafish imaging technology proved to be a key research tool in the study of hematopoietic emergence in this model system. Pioneering developers of this technology were present at the meeting (Bertrand et al., 2010; Kissa and Herbomel, 2010) and had used it previously to demonstrate that HSCs emerge directly from the aorta floor through a process called the endothelial to haematopoietic transition (EHT). These cells then migrate to the cardinal vein before finally homing to the caudal hematopoeitic tissue. Talks on this topic were focused on the use of imaging technology to understand the signals that drive HSC emergence. Karima Kissa (CNRS, Montpellier, France) showed preliminary data on how the mechanical forces shape the morphology of the aorta at the site of HSC emergence and recruitment of macrophages have a crucial role in the migration process from the aortic floor to the cardinal vein. Julien Bertrand (U. Geneva, Geneve, Switzerland) presented data on the role of TFEC, an important transcription factor expressed in vascular cells of the caudal hematopoetic tissue. Here, TFEC controls expression of several cytokines, including kitlgb. Mutants for the TFEC factor lose all their hematopoietic progenitors by 4 days post-fertilization and become anemic by 8-9 days post fertilization (Mahony et al., 2016). David Traver (UCSD, San Diego, USA), who previously showed a specific requirement for non-canonical Wnt in the somites to induce Notch activation in early blood developmental stages (Clements et al., 2011), discussed a temporal requirement for canonical Wnt signaling in the vasculature, specifically mediated by Wnt9a. He demonstrated that genetic and pharmacological manipulation of Wnt signaling affects the number of HSCs that emerge in the dorsal aorta.

The study of mouse embryonic hematopoiesis was the other important topic of the session. In the mouse model, HSCs are formed within hematopoietic c-kit+ clusters that emerge in the aortic endothelium in the E10-11 Aorta/Gonad/Mesonephros (AGM) region. Catherine Robin (Hubrecht Institute, Utrecht, The Netherlands) introduced a key conundrum on cluster heterogeneity by showing that only 2-3 HSCs are detected in one embryonic aorta, despite the fact that at least 600-700 cells form all the clusters. Interestingly up to 12 HSCs can be detected if maturation protocols are applied before transplantation into irradiated animals (Taoudi et al., 2008; Zhou et al., 2016), or if the cells are transplanted into the neonate fetal liver (Boisset et al.,

2015) indicating that some cells are pre-HSCs. Thus, discussion in this session concentrated on the composition of the hematopoietic clusters as well as how they generate HSCs, and the important signals coming from the AGM niche that may regulate this. Robin discussed how her lab had explored the molecular signatures of the successive populations leading to HSC production by using single cell RNA-sequencing. By taking advantage of the gfi1 reporter (Thambyrajah et al., 2016) in conjunction with classical markers, different subpopulations of cells were analyzed and compared at embryonic day 10 and 11.

The niche of nascent HSCs in the AGM was widely discussed. By using a combination of genetic reporters and cre lines, Vashe Chandrakanthan (a postdoctoral fellow in Pimanda's lab, UNSW, Australia) dissected different elements of the aortic niche based on the expression of Nestin and PDGFRA. He reported exciting results which demonstrated the induction of LT-HSC activity from E13.5 non-hemogenic endothelial cells by re-aggregation with E11.5 specific stromal populations. Another important element of the AGM niche is the sympathetic nervous system, previously described by Katrin Ottersbach, University of Edinburgh (Edinburgh, UK) (Fitch et al., 2012). In her talk, Ottersbach showed how embryos deficient for p57Kip2/Cdkn1c have an expanded sympathetic nervous system compartment, as detected by Gata3 expression, and explained how this translates into an increase of HSC production. This appeared to be a direct effect through the beta2-adrenergic receptor expressed on emerging HSCs.

In addition to new identified signals, one of the old players, the Notch pathway, was also covered in this session. Anna Bigas (IMIM, Barcelona, Spain) indicated that different levels of Notch activity are used to specify different tissues. By using two reporter mouse models developed in the laboratory of Raphael Kopan (Liu et al., 2015) that trace cells with different levels Notch-activation history, she showed how she could distinguish between two distinct Notch-dependent fates - arterial and hematopoietic - that originate from different precursor cells (Gama-Norton et al., 2015). Similarly, Michelina Iacovino (UCLA, Los Angeles, USA) presented data to suggest that Cis-inhibition of the Notch pathway downstream of HoxA3 could play a role in blocking blood development during embryonic stem cell (ESC) differentiation.

Finally, Thierry Jaffredo (CNRS-Inserm-UPCM, Paris, France) brought it all together with his inspiring Keynote Lecture on how different species have evolved to generate

HSCs and their associated niches. He presented his lab's research on the identification of the hemogenic endothelium using tracing techniques, ESCs and live imaging. Jaffredo also discussed recent work on the pre-somitic mesoderm as a source of endothelium, hemogenic endothelium and cells undergoing EHT (Yvernogeau et al., 2016). Some of the finer points on the formation of the dorso-ventral polarity of the aorta in the mouse, zebrafish, chick and human embryos – the size of the notochord and the influence of the Shh-VEGF axis, for example - and how it affects the polarization of HSC emergence were discussed.

Hematopoietic stem cells in the adult

The hematopoietic stem cell niche

A full day of the meeting was dedicated to talks on the localization of adult HSCs and how their differentiation is regulated. By integrating intravital microscopy, computational analysis of images, flow cytometry and mathematical modelling, Cristina Lo Celso (Imperial College London, UK) addressed how stresses such as infection or leukaemia influences normal blood cell generation in situ. Cristina showed that during infection HSCs become either motile (although not mobilized) or very still, and that the flux through hematopoietic stem and progenitor (HSPC) populations is likely dramatically altered (Vainieri et al., 2016). Furthermore, results presented by Delfim Duarte from her laboratory highlighted the increasing migratory behaviour of leukaemia cells from early bone marrow infiltration to overt chemoresistance, and their relation with bone marrow niches known to maintain HSCs (Hawkins et al., in press). In keeping with this theme, Paul Frenette from the Albert Einstein College of Medicine, New York, USA emphasized a complex interplay between niche cells and hematopoietic cells in the bone marrow under steady state conditions in the adult. Frenette also reported exciting data on HSC localization during development, where Nestin+ NG2+ pericytes that are associated with portal vessels provide HSC niches that support HSC expansion in the fetal liver (Khan et al., 2016). Interestingly, HSCs appear to relocate from the fetal liver to the bone marrow upon loss of these specialised pericytes.

Xenotransplantation of human HSCs into a mouse HSC niche is a critical tool for understanding how human HSCs interact with the niche and new strategies of transplantation without conditioning allows the study of this interaction under steady-state conditions (Cosgun et al., 2014). To this end, Claudia Waskow from the Technical University in Dresden, Germany presented data to show that human donor HSCs can communicate with murine niche cells upon transplantation, suggesting that

they shape their microenvironment according to their needs. It will be interesting to visualize HSCs in their complex environment once multicolour imaging becomes available. To this end, Timm Schroeder from the ETH Zürich in Basel, Switzerland discussed novel tools for long-term single-cell imaging and tracking (Hilsenbeck et al., 2016), and how they were used to quantify expression dynamics of transcription factors in differentiating HSCs. The results challenge the current model of myeloid lineage choice initiation by a PU.1-GATA1 stochastic switch (Hoppe et al., 2016).

Hematopoietic stem cell differentiation

A new concept regarding how steady-state hematopoiesis occurs in the adult mouse was presented by Katrin Busch from the Rodewald laboratory at the DKFZ in Heidelberg, Germany. She presented a self-generated elegant in situ fate mapping mouse tool that allows tracing of differentiating progeny that arise in the bone marrow from the most primitive HSC in situ (Busch et al., 2015). Most immature HSCs were only rarely involved in the daily generation of new blood cells, implying that steady-state hematopoiesis is largely independent of the activity of HSC over long periods of time. In contrast, despite the low contribution of individual HSCs, many HSCs participate in hematopoiesis under steady state - just at very low frequencies. This is in stark contrast to hematopoiesis in post-transplantation settings that relies on the activity of just a few clones.

How is HSC function regulated? Martijn Nolte (Sanquin, The Netherlands) provided insight into the regulation of HSC self-renewal by T cells, particularly by memory CD8+ T cells and also virus-specific memory CD8+ T cells, by a yet to-be-determined soluble factor. To uncover new regulators of HSC function Nina Cabezas-Wallscheid a postdoctoral fellow in Andreas Trumpp's laboratory (DKFZ and HI-STEM, Heidelberg, Germany) showed by bulk and single-cell RNA-seq analyses that the transition from dormancy towards cell cycle entry is not achieved by a simple binary on/off switch, but rather that it follows a continuous up-regulation of all major biosynthetic processes: transcription, splicing, translation, metabolism and so on. During dormancy, cells tune down these processes to a very low base level. The team also reported a novel transgenic reporter mouse that specifically labels dormant HSCs and thus avoids time consuming label retention assays to identify, isolate or characterize these extremely rare cells. One of the key regulators controlling entry and exit from dormancy in HSCs and in pluripotent cells of the pre-implantation embryo is the Myc oncogene (Laurenti et al., 2008; Scognamiglio et al., 2016). While

the JAK-Stat pathway controls Myc expression in pluripotent cells, data were provided suggesting that retinoic acid signaling is upstream of Myc in HSCs.

To better understand HSC commitment and differentiation and how these processes are regulated, Simon Haas from Marieke Essers lab (DKFZ and HI-STEM, Heidelberg, Germany) together with the Steinmetz (EMBL, Heidelberg / Stanford University) and Trumpp groups developed a novel multiplexed approach that integrates flow-cytometric, transcriptomic and functional lineage-fate data at the single-cell level. The data were used to characterize developmental transitions during HSC commitment and differentiation and point to a novel model for a continuous differentiation flow conceptualized by a 'developmental continuum'. The data sets were used to identify the molecular and cell biological processes, transcription factors and signalling pathways associated with lineage commitment and maturation. Adding an interesting metabolic angle to the story, Satish Khurana (The Indian Institute of Science Education and Research Thiruvananthapuram, India) used a comparative gene expression approach between fetal liver (FL)- and adult bone marrow-derived HSCs, to show that highly proliferative FL HSCs met their increased energy demand by upscaling the number and activity of mitochondria (Manesia et al., 2015). Importantly, niche-HSC interactions were retrieved by that screen and outside-in integrin signalling via integrin-av (Itgav) identified as a key pathway regulating the proliferation of HSCs. Consistent with these data, the loss of Itgav lead to increased HSC proliferation and loss of stemness in vivo (Khurana et al. Nat Com, in press). Continuing with the metabolic theme, Toshio Suda from National University of Singapore (NUS) (Singapore) gave an excellent keynote lecture about HSC metabolism, focusing on the difference between steady state and stress hematopoiesis with regard to HIF1a function and p38 signaling. He described how Folliculin (FLCN)-TFE3 axis is also involved in suppression of oxidative metabolism, and how FLCN-deleted mice show multi-abnormal phenotypes, which include bone marrow (BM) failure, abnormal function of macrophages (hemophagocytosis) and osteoclasts (resulting in osteoporosis) (Baba et al., 2016) These phenotypes might be explained by a common cause, which is the result of a hyper-oxidative metabolism and nucleic acid disturbance within the niche. He discussed how the metabolic state is not a result of cell status, but that it can cause changes cell differentiation and activity.

Hematopoietic stem cell aging

HSC aging was specifically covered in the meeting and introduced by **Emmanuelle Passague from the University of California, USA**, who discussed her work showing that autophagy protects HSCs from aging-associated functional decline (Warr et al., 2013). Passague showed how HSCs from aged mice display striking heterogeneity with respect to their autophagy levels, with high autophagy levels maintaining a low metabolic state and protecting the cell's intrinsic robust long-term engraftment activity. Hartmut Geiger (Cincinnati Children's Hospital Medical Center, Cincinnati, USA and University of Ulm, Germany) showed that inhibition of the small Rho GTPase Cdc42 activity rejuvenates HSC function. In fact, a shift from canonical to non-canonical Wnt signalling due to increased expression of Wnt5a in aged HSCs switches the aging process 'on', via activation of Cdc42. Consistent with this, Wnt5a haploinsufficiency and knock-down approaches both result in rejuvenation of HSCs (Florian et al., 2013). Subsequently, Novella Guidi, a graduate student from Geiger's laboratory presented exciting data on the critical role of stroma-derived osteopontin in HSC aging in vivo. A rejuvenating effect was observed following brief exposure to thrombin-cleaved osteopontin, suggesting that HSC aging, in fact, may be able to be reverted. Karl Lenhard Rudolph from the Fritz Lipmann Institute for Aging in Jena turned towards DNA damage control. Rudolph presented an update on his previous work showing that DNA damage limits the self-renewal of HSCs by inducing BATF-dependent lymphoid differentiation (Wang et al., 2012). His current work is now aimed at analysing the consequences of BATF deletion on hematopoiesis and leukemia formation in aging mice. Moving from aging to disease, Richard Groen (VU University Medical Center, Amsterdam,, The Netherlands) presented recent advances on his lab's efforts to build a bone marrow-like HSC niche using ossicles that were previously shown to be optimized tools for the engraftment of leukemic cells (Sontakke et al., 2016). Groen showed how adding human blood vessels to the bone marrow-like ossicle system resulted in improved human HSC, acute myeloid leukemia and multiple myeloma engraftment and differentiation as shown by xenotransplantation experiments into RAG2-/-gamma(c)-/- recipient mice.

New technologies for hematopoietic stem cell research

New technologies are providing new avenues to explore old questions in HSC biology. In particular, single cell RNA-sequencing (RNA-seq) has emerged as a powerful tool to decipher the trajectory of cells within a given differentiation pathway. To this end, Dana Pe'er (Columbia University, New York, USA) described the use of single cell datasets to map developmental trajectories and how these go awry in

disease. By taking a multi-dimensional approach, combining single-cell data from mass cytometry and RNA-seq,, she showed how it was possible to order cells according to their developmental progression and label each cell as pre-bifurcation or as one of two post-bifurcation cell fates (Setty et al., 2016). She highlighted the importance of distinguishing between the noise/artifact and biological signal in these high-throughput technologies, stressing that methods that do not account for this can be misleading. Once cells were aligned on a trajectory, she was able to follow the order of events during development, identify transitional populations as well as the populations that go awry in disease.

Single cell RNA-sequencing is an equally powerful tool in the study of developmental HSC biology, as it can be used to decipher the trajectory of embryonic cells. Bertie Gottgens (University of Cambridge, Cambridge, UK) reported a regulatory network model for hematopoiesis based on extensive experimental evidence and validated by single cell expression profiling (Schütte et al., 2016). He also demonstrated how ectopic activation of cardiac genes in TAL1/yolk sac endothelium is unlikely to be the immediate result of a binary fate switch (Scialdone et al., 2016). Taking a more global look at the regulatory dynamics of hematopoietic specification, Constanze Bonifer (University of Birmingham, UK) presented the work of a UK-wide consortium aimed at obtaining a comprehensive genome-wide map of transcription factor occupancy, chromatin accessibility, histone modifications and gene expression from a complete developmental pathway (Goode et al., 2016). To this end, the groups performed in vitro differentiation of mouse embryonic stem cells and purified the cells from 6 different stages of hematopoietic specification and differentiation. They were able to identify the dynamic gene regulatory networks that regulate the transition between these the different cell types, from mesodermal precursors to macrophages. One of the important pathways identified in the hemogenic endothelium was Hippo signalling which may be an important regulator in the early stages of hematopoietic differentiation. A relatively new and innovative approach to understanding gene expression at the four-dimensional level – that is, through both space and time – is “tomo-seq”, a genome wide RNA tomography approach. With the aim of identifying conserved molecular pathways involved in HSC production, Laurent Yvernogeau, (a postdoctoral fellow in Robin’s Lab, Hubrecht Institute, Utrecht, Netherlands) used a genome wide RNA tomography approach called “tomo-seq” (Junker et al., 2014). He collected and sequenced sequential sections of whole aortas (cut along the anterior-to-posterior

axis) and thick transversal embryo slices (cut along the ventral-to-dorsal axis) from various embryo species isolated at different developmental stages: before, during and after cluster/HSC emergence. This approach should provide an evolutionary 4D map of gene expression through space and time of development.

Lineage reprogramming may still be considered a relatively recent technology in the HSC field, and this meeting heard progress from two labs on this topic. Shahin Rafii, Cornell University, (Ithaca, USA) introduced an innovative reprogramming approach whereby endothelial cells (ECs) could be converted into bona-fide transplantable HSCs (Sandler et al., 2014). By employing Runx1-reporter mice, his group could distinguish and track the emergence of reprogrammed ECs into authentic HSCs, which were capable of serial and long-term multilineage repopulation, including the production of polarized T-cells. Data from reprogramming fibroblasts was presented by Carlos-Filipe Pereira (University of Coimbra, Portugal), who then used this information to isolate early precursor HSC cells from the mid-gestation placenta. Pereira characterized these cells by single-cell RNAseq and further showed that expressed Prom1, Sca1 and CD34, and that they localized to the placental vascular labyrinth at the maternal-to-fetal interface (Pereira et al., 2016)

Concluding remarks

The study of haematopoiesis has a long history and, although the field has come far, many of the key questions remain. One of the most exciting aspects of this EMBL meeting was the realisation that we may now be close to answering some of these questions definitively, thanks to recent advances in single-cell data collection and analysis, as well as new approaches to imaging. The majority of meeting participants agreed that these technologies will soon allow a clearer picture of hematopoiesis and leukemogenesis to emerge. There is no doubt that this is a unique and exciting time to be studying hematopoietic development, and we look forward to the discussion of future breakthroughs at the next EMBL meeting on hematopoiesis in 2018.

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