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Cellules souches du système vasculo-sanguin : émergence et auto-renouvellement / Stem cells of the blood/vascular system: emergence and self-renewal

Liste des participants

Anna Bigas, Chantal Cerdan, Marelle de Bruijn, Françoise Dieterlen-Liévre (Organisateur), Charles Durand, Elaine Dzierzak, William Fleming, Pascale Gaussem, Marc Haenlin, Tara Huber, Thierry Jaffredo (Organisateur), Julian Martinez Agosto, Alexander Medvinsky, Shin-Ichi Nishikawa, Roger Patient, Igor Samokhvalov, Nancy Speck (Organisateur), Mervin Yoder, Elias T. Zambidis, Leonard Zon.



Compte-rendu

Stem cells of the blood/vascular system: emergence and self-renewal

Organizers: Françoise Dieterlen-Liévre, Thierry Jaffredo, Nancy Speck
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Molecular control of hematopoiesis

Transcription

Leonard Zon. *Children's Hospital Howard Hughes Medical Institute Harvard, USA.*

Genetics of AGM Formation in the Zebrafish

Roger Patient *The Weatherall Institute of Molecular Medicine, Headington, UK.*

HSC emergence in the dorsal aorta in Xenopus and zebrafish

Marella De Bruijn. *Weatherall Institute of Molecular Medicine, Oxford, UK.*

Transcriptional regulation of Runx1 in developmental hematopoiesis

Marc Haenlin. *Centre de Biologie du Développement, Toulouse, France.*

Pervasive role of a GATA/RUNX cis-regulatory module in Drosophila blood cell commitment and differentiation

Signalling

Julian Martinez-Agosto *David Geffen School of Medicine, UCLA, USA.*

Genetic dissection of hematopoiesis using Drosophila as a model system

Ana Bigas *Molecular Oncology Center IDIBELL, Barcelona, Spain.*

Dissecting Notch functions in the mouse AGM hematopoiesis

Charles Durand. *Developmental Biology laboratory, UPMC, Paris, France.*

Molecular control of AGM-HSC activity

HSC and Endothelium: related lineages?

- Embryonic models.

Thierry Jaffredo *Developmental Biology laboratory, CNRS, UPMC, Paris, France.*

Dynamics of aorta formation and hematopoietic production viewed from the chick angle.

Alexander Medvinsky. *Institute for Stem Cell Research, University of Edinburgh, UK.*

Selective rescue of definitive haematopoietic stem cells in Runx1 knockout embryos

Nancy Speck *Dept of Biochemistry, Dartmouth Medical School, Hanover, NH, USA.*

Hematopoietic cell emergence occurs through vascular endothelium cadherin positive cells.

- **ES models**

Tara Huber *Genome Institute of Singapore*

ES- and mouse embryo-derived hemangioblasts - what have we learnt by studying these progenitors?

Elias Zambidis Johns Hopkins University School of Medicine, Baltimore, USA.

Developmental regulation of human embryonic hemangioblasts

Chantal Cerdan Stem Cell and Cancer Research Institute, Hamilton, Canada.

Human Embryonic Stem Cells: from undifferentiated state to hematopoietic differentiation

Sites of hematopoiesis: ongoing challenges.

Mervin Yoder *Wells Center for Pediatric Research, Indiana, USA*

Yolk sac hematopoiesis: more than meets the eye.

Igor Samokhvalov *Riken Center for Developmental Biology, Japan.* The yolk sac reloaded

Elaine Dzierzak *Erasmus University Medical Center, Rotterdam, The Netherlands.*

Differential regulation of HSC in extra- and intra-embryonic sites

Alexander Medvinsky *Institute for Stem Cell Research, University of Edinburgh, UK.*

So, what is the source of HSCs in the embryo?

Nancy Speck *Dept of Biochemistry, Dartmouth Medical School, Hanover, NH, USA.*

Hematopoietic potential of the mouse allantois

Shin-Ichi Nishikawa *Riken Center for Developmental Biology, Japan.*

A new model for development of hematopoietic stem cells.

Françoise Dieterlen-Lièvre *Institut Alfred Fessard Gif sur Yvette, France.*

Ontogenic commitment of HSCs and endothelial progenitors: a moveable event.

Bi-potential circulating progenitors

Pascale Gaussem *Faculté de Sciences Pharmaceutiques et Biologiques, Paris, France.*

endothelial progenitor cells: definition and prospects for autologous cell therapy

Mervin Yoder *Wells Center for Pediatric Research, Indiana, USA*

Circulating and resident human endothelial progenitor cells

William Fleming *Center for Hematologic Malignancies, Portland, USA*

Endothelial cell potential of hematopoietic progenitors.

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Blood cells in the adult turn over constantly. Their renewal depends on hematopoietic stem cells that become segregated during development. The circumstances of this segregation have been the subject of active investigations for many years, leading to the conclusion that this event occurs during a protracted period of embryogenesis in various sites, both extra- and intra-embryonic, and may involve a bi-potent progenitor, the hemangioblast. In view of the importance of understanding the mechanisms of stem cell determination and maintenance, which are hoped to open important therapeutic perspectives, the colloquium addressed the many questions still pending at the cytological and molecular levels. Vertebrate and invertebrate models were compared and some clinical advances were described.

The first topic was the molecular control of hematopoiesis. Len Zon pointed out that hematopoiesis in the aortic region of the zebrafish embryo (the so-called AGM) is very similar to that in higher vertebrate species. Using zebrafish genetics, his group has identified ten independent genes that are required for hematopoietic stem cell formation in this region, in particular *Mindbomb*, whose mutation affects the Notch pathway, and the phospholipase *Cg* gene, whose mutation affects VEGF signalling. Multiple signalling pathways were found to interact during emergence of aortic blood stem cells. A chemical genetic screen reveals for the first time a major role of prostaglandins in hematopoiesis, through a cyclic AMP step. This striking finding was extended to mice in which PGE2 amplified the number of bone marrow progenitors and/or Hematopoietic Stem Cell (HSC) *in vitro* and *in vivo*. This discovery is likely to have clinical relevance, for instance cord blood HSC could be amplified before reconstitution, since the number of HSC in cord blood is limiting in the treatment of adults.

Roger Patient reported HSC emergence of HSC in the dorsal aorta of *Xenopus* and zebrafish embryos. Exploiting the experimental advantages of these two models, Dr. Patient's group elucidated transcription factors and embryonic signals that programme HSCs. They have established the hierarchical contributions of the transcription factors, *Fli1*, *Tel1*, *Scl*, *Lmo2* and *Runx1* and characterised sequential activities of hedgehog, VEGF, notch and BMP. The contributions of most of these regulators differ in adult HSC and embryonic blood programming.

Marella de Bruijn is characterizing the critical cis-acting regulatory sites in the transcription factor *Runx1/AML1* gene, which encodes an important regulator of hematopoiesis, required for the generation of the first definitive HSC in the major vasculature of the mouse embryo. Though a pivotal factor, its transcriptional regulation was still largely undefined. Combining comparative genomics and chromatin analysis, M. de Bruijn has identified a highly conserved 531-bp enhancer located at position +23.5 in the first intron of the 224-kb mouse *Runx1* gene, which is important for the early hematopoietic expression of *Runx1*. The recruitment of *Gata2*, *Ets*

SCL/Lmo2/Ldb-1 transcription factors to this enhancer is critical for initiating HSC generation.

Marc Haenlin and Julian Martinez-Agosto discussed the common transcriptional networks and signalling pathways shared between *Drosophila* and vertebrate hematopoiesis. They first described the characteristics of the *Drosophila* blood system which consists of three myeloid-like cell types. The simplicity of this model system allows the dissection of a binary switch in hemocyte specification. According to Dr. Martinez-Agosto, the hemangioblast concept is valid in *Drosophila*, since a single precursor gives rise to both blood and cardioblast cells (the vascular cell type in *Drosophila*). This investigator also described a population of blood progenitors that is maintained under the control of a niche in the larval hematopoietic organ, providing a powerful model to unravel the molecular mechanisms of stem cell maintenance, which may also be operational in vertebrate hematopoietic niches. Given the new tools available in *Drosophila*, Dr. Haenlin described an RNAi-based screen to identify genes controlling homeostasis of the hematopoietic niche.

Ana Bigas reported that Notch is required for the initiation of the definitive hematopoietic programme in the mouse embryo. In the yolk sac however, it is dispensable since all hematopoietic cell types and progenitors are found in Notch mutant embryos from different species. The Notch pathway does, all the same, control erythroid homeostasis by inducing apoptosis both in yolk sac and bone marrow. *GATA2* is directly regulated by Notch in murine hematopoietic cells.

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Charles Durand reported a characterization of the stem cell microenvironment in ontogeny. In the mid-gestation mouse embryo, the AGM (aorta-gonad mesonephros) region autonomously generates the first HSCs and serves as an HSC-supportive microenvironment. His talk focused on an experimental strategy that allowed the identification of novel regulators of AGM HSC activity, in particular the BMP pathway, which he showed to be critical for the emergence of HSCs from the AGM.

The next topic was the *in vivo* exploration of developmental relationships between endothelium and HSC in the aorta. Thierry Jaffredo described a dynamic *in situ* analysis of the expression patterns of hematopoietic and endothelial specific genes in the avian embryo. The Runx1 transcription factor is one of the earliest markers of the prospective hemogenic endothelium. Expression of Runx1 is followed by that of other hematopoietic markers such as PU.1, Myb, and CBF β while expression of endothelial markers such as VE-cadherin is reciprocally downregulated. Tagging of the endothelium one day before cluster emergence resulted in the formation of tagged hematopoietic clusters. Orthotopic transplantations between chick and quail embryos showed that the ventral endothelium of the dorsal aorta, which is hemogenic, disappears as hematopoietic production occurs and is replaced by somite-derived endothelial cells, which originate from the dermomyotome. On the other hand it is the sclerotome that gives rise to the smooth muscle cells of the aortic wall.

Nancy Speck showed that disruption of Runx1 in VE-cadherin positive cells of the mouse embryo blocked the emergence of both HSCs and hematopoietic progenitors. Preliminary data suggest that the requirement for Runx1 ends once the cells express Vav. In a reciprocal experiment, Alexander Medvinsky showed that rescue of Runx1 function limited to Tie2 positive cells was sufficient to rescue the defect. A discussion ensued about whether all hematopoietic stem cells develop from endothelial cells. The general consensus was yes, although there was some disagreement about how one defines an endothelial cell. Finally, William Flensing described his efforts to identify the adult equivalent of a hemogenic endothelium. He reported that although adult endothelial cells could provide impressive radioprotection, they did so by stimulating hematopoietic recovery of host cells. At present, no one has identified a convincing adult equivalent of the hemogenic endothelium, although its existence remains possible.

Mervin Yoder re-investigated the formation of the yolk sac. The classical depiction of extraembryonic mesoderm migrating from the posterior primitive streak and into the proximal yolk sac region with subsequent differentiation into blood cells (those in the middle of the mesodermal mass) and endothelial cells (those in the outer edge of the mesodermal mass) is not an accurate description of the cellular events of blood island organogenesis. Dr. Yoder's group, using confocal microscopy on precisely staged embryos treated with batteries of monoclonal antibodies, determined that extraembryonic mesoderm specification to primitive erythroid progenitor and angioblast cells is nearly coincident with the emergence of mesoderm from the primitive streak. Primitive erythroid and angioblast cells migrate independently into the proximal yolk sac where massive erythroblast proliferation first occurs extravascularly inside a unique prominent blood band. Just prior to cardiac contraction onset, the enveloping endothelial cells break up the primitive erythroblast mass into blood islands. Subsequently, the erythroblasts and CD41 high definitive progenitor cells (that have budded from yolk sac endothelium) leave the engorged blood islands of the proximal yolk sac and fill the luminal void of the capillary plexus that empties into the beating heart, distributing cells throughout the embryo proper. Further studies, using an *Ncx1* null mouse in which no circulation develops, have provided evidence that essentially all the definitive progenitor cells that seed the fetal liver at the 28 sp stage are products of the yolk sac. Additional evidence was presented that B lymphoid cells emerge autonomously and mature within the yolk sac and PSp of the *Ncx1* null embryo.

Igor Samokvalhov presented provocative data on the origin of HSC from the yolk sac. In order to directly analyze the embryonic sources of the lineage, he performed a conditional genetic cell tracing. The *Runx1* gene, which is critical for adult hematopoiesis, was used as a platform to direct the expression of a MER-Cre-MER hybrid protein, which supposedly allows specific labelling of *Rosa26R* knock-in yolk sac cells when tamoxifen is administered. With this system, Dr. Samokvalhov reports a significant contribution of early yolk sac cells to adult hematopoiesis, lack of hemangioblasts in embryonic day 7.5 (E7.5) yolk sac blood islands, and completion of HSC lineage specification by E9.5. His interpretation is that vascular beds, in particular those in the AGM, are colonized by blood-borne cells of yolk sac origin; he thus negates the occurrence of independent emergence of HSC in the AGM region, despite the experimental findings demonstrating the independent emergence of HSC in the AGM region, as described by all other participants. It should be emphasized that, since tamoxifen is injected into the mother, the labelling time course is difficult to assert precisely and could vary between 6 and 48 hours after the injection.

Eaine Dzierzak then discussed the local inducing microenvironment in the mouse aorta-gonad-mesonephros (AGM) region. The temporal appearance of the first adult repopulating HSC activity in the midgestation mouse AGM coincides with the appearance of hematopoietic clusters closely associated with the endothelial lining of the dorsal aorta. These HSCs express Ly-6A GFP and Runx1, and are localized to the hematopoietic and endothelial lineages. Most clusters are dependent upon the expression of the GATA-2 transcription factor. Chimeric explant cultures indicate that tissues dorsal to the AGM suppress, while tissues ventral to the AGM enhance hematopoietic progenitor and stem cell activity, indicating the importance of local microenvironments.

The next session, dedicated to the Embryonic Stem cell (ES) model, described the state of the art concerning the generation of hematopoietic and endothelial derivatives from mouse and human ES cells.

Tara Huber, using the mouse embryonic stem (ES) cell differentiation system, could isolate the *in vitro* equivalent of a hemangioblast, i.e., a cell with both hematopoietic and vascular potential. In the methylcellulose-based blast colony forming assay, a key stage in hematopoietic development (BI-CFC for Blast-Colony Forming Cell) was defined. Similar progenitors were isolated from embryo

between the early gastrulation till the 20 somite pair stage (E7-8.5). Two hemangioblast populations were found with different hematopoietic and vascular potential and reflect the location and developmental stage at which they are isolated, namely the early hemangioblast (yolk sac potential in the early population and definitive hematopoietic program expected from the P-Sp/AGM in the E8.25 hemangioblast). Using cells harboring eGFP cDNA knocked into the brachyury locus it was shown in both the ES system and the mouse embryo that the hemangioblast is a Flk-1+Brachyury+ cell. Through study of the hemangioblast a paradigm was provided for studying the establishment of other mesodermal tissues such as the cardiovascular progenitor which has both cardiac and vascular potential. These progenitors are Flk-1+Brachyury+ cells, they arise later than hemangioblasts during embryoid body differentiation; thus differentiation in EBs intriguingly recapitulates the orderly generation of hematopoietic and then cardiac mesoderm in the embryo. The findings from the mouse have been translated to the human ES cell (hESC) system, showing that hemangioblasts arise during *in vitro* differentiation of hESCs, thus extending the study of embryonic hematopoietic commitment to human cells.

Elias Zambidis described serum-free culture conditions for efficiently generating clonogenic hemangioblasts from pluripotent human ES cells (hESC). He also reported that angiotensin-converting enzyme (ACE), a critical physiologic regulator of blood pressure, angiogenesis, and inflammation, can be used as a marker for identifying and purifying 'hemangioblastic stem-progenitors' differentiating from hESC. Remarkably, the kinetics of YS-like hematopoiesis generated from hEB cells paralleled the developmental timetable of human YS, during weeks 2 to 6 of gestation. He also demonstrated that ACE and the renin-angiotensin system (RAS) axis play a direct role in hESC-derived hemangioblastic differentiation through the two major angiotensin receptors (AGTR1 and AGTR2). (Directed differentiation of hemangioblasts toward either endothelium, or multipotent primitive hematopoietic progenitors could be efficiently enhanced via modulation of AGTR1 vs. AGTR2 signaling.) RAS axis manipulation may direct the differentiation of transplantable hESC-derived hemangioblastic stem-progenitors, thus providing novel opportunities for human tissue engineering. The key molecular and cellular events of human stem-to-endothelial genesis can thus be delineated in a manner previously impossible, human embryonic tissue being difficult to obtain.

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Chantal Cerdan demonstrated robust hematopoietic differentiation from clusters of human ESCs (embryoid bodies or EBs) in the presence of stem cell factor (SCF), FLT3L, and BMP-4 (previously identified as required for maintenance and differentiation of mouse adult hematopoiesis). All hESC lines consistently displayed emergence of hematopoietic cells at day 10 of EB treatment. Detailed phenotypic analysis of cells in EBs prior to day 10 indicates that cells with endothelial phenotype and potential develop. To denote the absence of CD45 and expression of PECAM-1, Flk1, and VE-cadherin, these cells are designated as CD45negPFV. Prospective and clonal isolation of these cells demonstrated that this unique population is exclusively responsible for hematopoietic progenitors derived from hESCs and possesses "hemangioblastic" properties. However, the *in vivo* NOD/SCID repopulating potential of hematopoietic progenitors derived from CD45negPFV cells has been found to be very limited compared to adult sources of conventional HSCs. Molecular profiling uncovered surprising differences in expression of both homing molecules and HOX gene clusters, suggesting the inability of hESCs to activate essential genetic programs required to provide *bonafide* somatic-like HSCs. Since multi-lineage developmental potential of stem cells is not autonomously achieved, she characterized the cellular interactions and signaling required to maintain hESC cultures, using proteomic identification. Her study exposed a central and direct role for the IGF-1/IGF1R axis while the action of FGF is primarily indirect, through the induction of supportive (survival) factors such as TGF β , acting as a paracrine signal between hESCs.

Shin-ichi Nishikawa proposed a novel differentiation pathway of HSC. While endothelial cells can be derived from diverse mesodermal cell populations, hematopoietic cells are entirely derived from lateral mesoderm. This lateral mesoderm may diverge into three VE-cadherin expressing pathways, i) Runx1+GATA1+, ii) Runx1+GATA1-, iii) Runx1-GATA1- populations. Runx1 expression, essential for endothelial/hematopoietic transition, is regulated by external signals such as BMP4. Under normal circumstances, YS EC cannot give rise to hematopoietic cells due to a low concentration of BMP4, although some YS EC have such a potential. The fate of VE-Cad+/Runx1+/GATA1+ cells is restricted to EC and HC, thus defining the hemangioblast. Some cells from this population migrate and eventually give rise to the definitive HSC. While it is possible to induce this particular stage in ES cell differentiation culture, the conditions to induce the definitive HSC are yet to be determined.

Françoise Dieterlen-Lièvre reported that, while diverse compartments of the mesoderm contribute to the construction of the blood-forming system, they display very distinct potentials in that regard, as shown in the avian model. The splanchnopleural mesoderm, which is intimately associated with endoderm, has a hemangioblastic potential, i.e., gives rise to both endothelial and blood cells. This dual potential is expressed in the embryonic appendages, which are composed of mesoderm and endoderm, namely the yolk sac and the allantois. The latter appendage first recognized as a hemopoietic organ in the avian model, was shown recently to give rise to hematopoietic progenitors in the mouse embryo. In the embryo proper the only site where this potential appears to be expressed is the floor of the aorta. The somatopleural mesoderm, associated to ectoderm, does not contribute any cell type to the blood-forming system (nor does the amnion, the appendage composed of the same germ layers) while the somites give rise to a population of endothelial cells responsible for vascularizing the body wall, limbs, bone, skin, that is all the derivatives of the somatopleure. Some growth factors, probably involved in inducing or maintaining these potentials, were discussed.

Mervin Yoder discussed the Endothelial Progenitor Cells (EPC), defined as marrow-derived cells that circulate in the bloodstream and display postnatal vasculogenic activity. However, good assays for these cells have been lacking. The group reported that the commercially available CFU-Hillr assay fails to identify endothelial progenitor cells, but instead, identifies myeloid progenitor cells differentiating into angiogenic macrophages *in vitro*. These macrophages, while displaying many of the cell surface phenotypic properties of an endothelial cell, retain primary macrophage characteristics such as phagocytosis of bacteria, expression of nonspecific esterase, limited proliferative capacity, and inability to spontaneously form vessels *in vivo*. In contrast, a rare circulating cell that gives rise to endothelial cells with clonal endothelial colony forming ability and forms human vessels that anastomose with murine vessels upon implantation to participate in the systemic circulation of the host, demonstrates those properties that define an EPC. He proposed that myeloid progenitor cells participate in neoangiogenesis, initiating the processes inherent to angiogenesis, but that the rare circulating endothelial colony forming cell and similar colony forming cells residing in the endothelial monolayer of venous and arterial blood vessels are the ones that actually provide the endothelial sprouts that form the new vasculature. Further studies will be required to ascertain the specific roles that myeloid cells and EPC play in normal and pathologic neoangiogenesis.

Pascale Gaussem described how injection of autologous hematopoietic stem cells or endothelial progenitor cells (EPC) expanded *ex vivo* improves neo-vascularization in adult patients, however the identity of the cell population responsible for these clinical benefits remains controversial. Her presentation summarized the clinical trials of angiogenic therapy, the definition of EPC, a method of *ex vivo* expansion by activation of the thrombin receptor PAR-1, and current methods of EPC quantification.

The meeting was very exciting, many novel data came out, controversial issues were discussed in depth, agreements were reached on many, while a few gave rise to heated debate.