MEETING REPORTS

IFSC 2020 Meeting Report

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Introduction

The 7th International Forum on Stem Cells (IFSC) in Tianjin, China, lived up to its promise to present an exciting program ranging from basic biology to translational research, encompassing hematopoietic stem cells and the minor populations of stem cells that exist in many tissues. Held virtually on November 18-19 because of the COVID pandemic, the meeting provided a timely opportunity for investigators to interact and share ideas with colleagues from around the world.

The meeting was organized by Professor Tao Cheng, who is currently Professor of Medicine at Peking Union Medical College (PUMC) and the Chinese Academy of Medical Sciences (CAMS), President of the Institute of Hematology & Blood Diseases Hospital at CAMS, Director of the State Key Laboratory of Experimental Hematology, as well as the founding Chairman of the Department of Stem Cell and Regenerative Medicine at PUMC, the founding Director of the Center for Stem Cell Medicine at CAMS, and the founding President of the Chinese Association for Blood Sciences (CABS).

Professor Cheng received his medical degrees from Second Military Medical University in Shanghai, China (1981-1989). He did his residency in internal medicine and clinical fellowship in hematology at Changhai Hospital, Shanghai, China (1989-1993). He received his postgraduate research training at the Hipple Cancer Research Center, Dayton, Ohio, and Massachusetts General Hospital, Boston, Massachusetts (1993-1997). He became an instructor in medicine in 1998, then an Assistant Professor in 2001 at Harvard University, an Associate Professor with tenure (2006-2010) and later a full Professor at the University of Pittsburgh School of Medicine (2010-2013).

Professor Cheng's laboratory primarily focuses on both genetic and epigenetic mechanisms in hematopoietic stem and progenitor cells, with each project guided by approaches intended to elucidate basic principles and develop practical strategies. Leukemia and bone marrow failure are the main diseases against which research advances in the laboratory are achieved. Through broadly collaborative approaches, Prof. Cheng is also committed to training hematologists and stem cell biologists and to building strong hematopoietic and stem cell research programs that will ultimately benefit the patients. Prof. Cheng is the founding editor-in-chief of Blood Science, the first English fundamental hematology journal in China. He has been on the editorial board of several leading journals in hematology and stem cell research including Blood, Leukemia, Experimental Hematology (Associate Editor), and International Journal of Hematology (Associate Editor).

On both days of IFSC 2020, the proceedings began with a keynote speech by an eminent investigator, followed by scientific sessions consisting of 3 or 4 talks grouped around a central theme. Video recordings of the meeting presentations can be found online at https://www.sciconf.cn/m/lives/details/2264?lang=en

DAY 1 KEYNOTE SPEECH

Professor Stuart Orkin

Introduced by Professor Tao Cheng

IFSC 2020 was opened by the Director of the State Key Laboratory of Experimental Hematology, Professor Tao Cheng, who welcomed delegates and introduced the first Keynote Speaker, Stuart Orkin, who received a BS from MIT and an MD from Harvard Medical School and is currently the David G. Nathan Distinguished Professor of Pediatrics at Harvard Medical School. Previously he served as Chairman of the Department of Pediatric Oncology at the Dana-Farber Cancer Institute from 2000-2016. Prof. Orkin served on the National Research Council Committee on Mapping the Human
Genome and as co-chair of the Panel to Assess the NIH Investment in Gene Therapy. He was the inaugural chair of the Grants Reviews Committee of the California Institute of Regenerative Medicine (CIRM).

Professor Orkin was elected to the National Academy of Sciences (NAS), National Academy of Medicine (NAM), American Academy of Arts and Sciences, and the American Philosophical Society. He received the E. Mead Johnson Award, Warren Alpert Prize, Helmut Horten Foundation Prize, Distinguished Research Award from the Association of American Medical Colleges (AAMC), E. Donnell Thomas, Dameshek and Basic Science Mentor Awards of the American Society of Hematology (ASH). He received the Jessie Stevenson Kovalenko Medal of the NAS for “important contributions to the medical sciences” (2013), the William A. Allan Award of the American Society of Human Genetics (2014), the George M. Kober Medal of the American Association of Physicians (2018), the Mechthild Esser Nemmers Prize in Medical Science of Northwestern University (2018), the King Faisal Prize in Medicine (2020), and the Harrington Prize for Innovation in Medicine (2020).

Remembrance of the things past: Turning on fetal hemoglobin to treat hemoglobin disorders for therapy

Professor Orkin began his interesting and highly informative talk with a quotation from Marcel Proust:

The only real voyage of discovery consists not in seeking new landscapes, but in having new eyes.

The quotation was prescient and proved to be a recurring theme throughout the meeting.

During his long and distinguished research career Prof. Orkin has provided the first comprehensive molecular dissection of an inherited disorder (the thalassemia syndromes), identified the first hematopoietic transcription factors (the GATA family), and characterized their roles in blood cell development and cancer. His studies of BCL11A, a repressor of fetal hemoglobin (HbF) have clarified regulation of globin gene switching and paved the way for HbF reactivation as therapy for the thalassemia and sickle cell disease.

In his inspirational presentation Prof. Orkin discussed how the detailed mechanistic understanding of the critical switch from fetal (HbF) to adult-type (HbA) is regulated during development has led to definitive therapy for patients with sickle cell disease (SCD) and beta-thalassemia.

Professor Orkin recalled that decades ago it had been hypothesized that reactivation of Hbf in adult erythroid cells would lessen the severity of the hemoglobin disorders. A formidable obstacle to this strategy was the lack of knowledge regarding how Hbf is silenced in the fetal to adult transition. Beginning with GWAS in 2007/8, the Orkin group focused attention on BCL11A, a zinc finger protein encoded on the short arm of chromosome 2. In a series of studies, they demonstrated that BCL11A is a major repressor of gamma-globin gene expression and its knockout in erythroid cells leads to robust reactivation of gamma-globin transcription and hence Hbf production. Such reactivation is sufficient to rescue the SCD disease phenotype in engineered mice. Further, they showed that common genetic variation detected in GWAS resides in an erythroid-specific enhancer within the BCL11A gene and the enhancer is essential for BCL11A expression in the lineage. Through comprehensive CRISPR/Cas9 mutagenesis, they identified a discrete region of the enhancer encompassing a critical GATA-site that is required for a major portion of its activity. This region has become the target of current gene editing trials using both zinc-finger nucleases and CRISPR/Cas9. Detailed chromatin binding studies employing CUT&RUN and gene editing revealed that BCL11A acts directly within the gamma-globin promoter to repress expression and does so through displacement of a ubiquitous activator, NF-Y. To achieve maximum repression, BCL11A recruits the NuRD corepression complex to the promoter and acts in concert with a second repressor, LCR, that binds further upstream in the gamma-globin gene promoter. The detailed mechanistic understanding of Hbf silencing has provided the foundation for ongoing clinical trials directed at BCL11A, using erythroid-specific shRNA, gene editing of the BCL11A enhancer, and destruction of the BCL11A binding site in the gamma-globin promoter. Preliminary results of clinical trials validate the preclinical science and have shown very promising benefit in SCD and beta-thalassemia.

The vision going forward is to achieve pharmaceutically what can now be done through genetics. In order to reduce the global burden of the disease significantly, effective pharmacological management will be needed viz a novel drug. This will require the development of a small molecule drug capable of inducing significant Hbf in vivo by targeting specific components central to Hbf repression, and for this BCL11A is an excellent target. Taken together, the work illustrates how fundamental insights into developmental control can now be translated for definitive therapy of patients with life-threatening disease.

Questions for Stuart Orkin

Lee Grimes: While you have suggested that the research in this arena will likely drive toward a small molecule drug, do you have any favorites in the gene therapy approaches such as forced expression of globin (such as that of Punam Malik) before we get the small molecule?

Stuart Orkin: The more irons in the fire the better. I think that fetal globin (HbF) expression (which is the approach that has been used by Bluebird Bio, Punam Malik at Cincinnati Children’s, and Don Kohn at UCLA) certainly can work. Because of the way the LCR interacts with the downstream genes, the
advantage of HbF reactivation is that if you reactivate the gamma gene, you shut off or downregulate the beta chain product, so you maintain proper physiologic regulation of the globin chains, at least theoretically. We think that is important. Whether it will matter I think only the trials can show. My suspicion is that all the approaches are beneficial. I think there is no doubt about that from the emerging clinical trials. Whether one is preferable to another, I think one needs more evidence.

Another question is to what extent is there a risk of lentiviral insertional mutagenesis? How does one weigh that up against off-target or chromosomal rearrangements from CRISPR/cas9? And then one has to weigh that against naturally occurring mutations that we all have as we age, so we have the baseline mutational rate and the invasive modifications to the genome. So, there are lots of different strategies, and I think we will see base editing as another strategy. We are entering a new era, and I think there will be many opportunities for patients.

Linheng Li: What is the natural reason behind the fetal hemoglobin switch to adult hemoglobin? If you reactivate the fetal hemoglobin in a patient, will it have any negative effects?

Stuart Orkin: The teleological reason is that fetal hemoglobin has a higher oxygen affinity which is beneficial to the fetus to draw oxygen from the mother's circulation. Fetal hemoglobin has different oxygen affinity to adult hemoglobin but the differences are relatively small especially compared to the differences between embryonic and adult hemoglobin. There are rare individuals who have 100% fetal hemoglobin because of large deletions of the beta globin cluster. They are exceedingly rare individuals but we have seen some that are heterozygous, they have 100% fetal hemoglobin. They are entirely normal, the mothers can in fact have offspring perfectly normally. So, although in a single generation it does not matter, maybe over the long haul of evolution it will be different. If we could all have 100% fetal Hb it would not be so bad.

Subsequently, on Day 1, fourteen speakers contributed to scientific sessions on four themes.

Stem Cell Gene Therapy
Chaired by Lee Grimes and Linheng Li

Programming and reprogramming of aging

Professor Guang-Hui Liu from the Institute of Zoology, Chinese Academy of Sciences, studies the mechanisms underlying human stem cell aging. His laboratory seeks to identify factors that affect human stem cell aging and to develop novel therapeutic interventions for the goal of “healthy aging”. Dr. Liu has published more than 100 publications in Nature, Science, Cell, and other prestigious journals. Dr. Liu has been an active member of the international scientific community; he is the president of the Chinese Society of Aging Cell Research (CSACR), the Deputy Editor-in-Chief of Protein & Cell, an Associate Editor of Stem Cell Research & Therapy, and an Editorial Board member of Cell Reports and Aging Cell.

Professor Liu explained that during the aging process, stem cells undergo functional decay and exhaustion, leading to compromised tissue regeneration and organismal aging. To promote tissue regeneration the Liu group have generated genetically enhanced stem cells and vascular cells with improved efficacy and safety by editing longevity genes and tumor suppressors. They envision that this approach could eventually be used to ameliorate chronic disease.

Questions for Guang-Hui Liu

Lee Grimes: You implicate the circadian transcription factor CLOCK in your analyses. Do you think the regeneration of hMSC was controlled by CLOCK, and if so, what is the mechanistic insight beneath that observation?

Guang-Hui Liu: Aging seems to be linked to changes in the circadian clock; however, we previously did not understand whether deregulation of circadian transcription factors underlies aging. We found that CLOCK decreases with aging, and that restoration of CLOCK using lentivirus expression vectors rejuvenates hMSC. Surprisingly, the transcription factor function of CLOCK is not requisite for this biology. Instead, CLOCK complexes with nuclear lamina and KAP1 to maintain heterochromatin architecture.

Lee Grimes: You performed single cell RNA sequencing of aging cells. How do you think that the aging-associated transcriptional changes are controlled at the epigenetic level?

Guang-Hui Liu: The single cell sequencing reveals cell type-specific gene expression changes at single cell resolution. These changes may be controlled, at least in part, at epigenetic levels.

Linheng Li: FoxO, as you identified to be an aging factor, may play different roles in different tissues; for example, FoxO is required for maintaining the quiescent hematopoietic stem cells in bone marrow; if you reduce the FoxO expression overall, it may extend some tissues’ life span but at the same time it may affect other tissue homeostasis.

Guang-Hui Liu: This is a good question, we need to take this as a consideration and thinking about tissue specific inhibition of aging factors including FoxO.

BCL11A enhancer editing for the beta-hemoglobin disorders

Dr Yuxuan Wu trained in the laboratories of Jinsong Li in the Chinese Academy of Sciences and Daniel Bauer in Harvard Medical School, where he studied pluripotency of embryonic
stem cells, CRISPR mediated genome editing in hematopoietic stem cells.

Dr Wu’s research program focuses on understanding the molecular mechanisms that regulate self-renewal and differentiation of hematopoietic stem cells (HSCs) and CRISPR mediated gene editing in human HSCs for sickle cell disease (SCD) and beta thalassemia. Returning to the theme of Professor Orkin’s lecture, Dr Wu reported on his work editing the BCL11A enhancer as a strategy for re-expression of the paralogous gamma-globin genes to produce HbF, which ameliorates sickle cell disease (SCD) and beta-thalassemia.

Dr Wu demonstrated that Cas9:sgRNA ribonucleoprotein mediated cleavage within a GATA1 binding site at +58 BCL11A erythroid enhancer resulted in highly penetrant disruption of the motif, reduction of BCL11A expression, and induction of HbF. The erythroid progeny of the edited SCD HSCs expressed therapeutic levels of HbF and resist sickling, while those from beta-thalassemia patients showed restored globin chain balance.

Questions for Yuxuan Wu

Linheng Li: As you presented that the editing efficiency in HSCs is very low, given that real HSCs within CD34+ cells is much lower, how can you sure you target HSCs.

Yuxuan Wu: Our previous report showed that our editing efficiency in CD34+ cells was 80%, and after transplantation, 4 months later, the editing efficiency reduced to 40% in the bone marrow of some mice. Then we were able to increase the editing efficiency up to 95% in CD34+ cells after optimization of the editing system, so that we can get efficient editing of the LT-HSCs to support long term engraftment by analyzing the indel rates of mice bone marrow 4 months after transplantation.

Transform cell and organ therapy using genome editing

Dr Luhan Yang is CEO of Qihanbio, a biotech company specializing in the development of human-compatible organs, tissues, and cells. Her group harnesses gene-editing technology, particularly CRISPR/Cas9, to exploit xenotransplantation in attempts to contribute to the global need for human organs for transplantation. Recently Dr Yang was honored in the Bloomberg 50 (2017), named “Young Global Leader” by the World Economic Forum (2017), and was featured in “30 Under 30” in Science and Healthcare by Forbes Magazine (2014).

The long-term strategy of Qihanbio is to use high throughput, multiplexable genome editing in combination with expertise in transplantation immunology to create immunologically privileged allogeneic organs for use as therapies to treat cancer, organ failure, and other medical conditions.

Questions for Luhan Yang

Linheng Li: As the immune system is very important for the human system, so in the immune privileged situation; how do you balance the immune system role for the organ maintenance and tumor surveillance?

Luhan Yang: Actually, we do not know; it needs more tests and also depends on the patient situation. But we will monitor it.

Lee Grimes: You show an amazing array of iPSC with several mutations introduced by CRISPR. That technology has off-target effects, suggesting that you would need to do single cell cloning; however, I do not think that iPSCs clone efficiently. How did you control for the off-target mutations?

Luhan Wang: Of course, many edited iPSC clones are abnormal and we need to get rid of them. In my postdoctoral studies, I established a method to efficiently perform single cell cloning of iPSC, and that is what we are using now.

Stem Cell Diseases and Target Therapy

Chaired by Nadia Carlesso and Jianxiang Wang

Bcl-xl PROTAC: A safer and more effective therapeutic agent for hematologic malignancies

Dr Daohong Zhou holds professorial positions in both the Department of Pharmacodynamics and Department of Radiation Oncology at the University of Florida at Gainesville. He serves as the Associate Director for Translation and Drug Development and the Harry E. Innes Endowed Professor of Cancer Research at the UF Health Cancer Center. His research has contributed to a better understanding of the role of cellular senescence in ionizing radiation and the efficacy of senolytic drugs, agents that selectively induce apoptosis of senescent cells. His work led to the discovery of the first potent and broad spectrum senolytic agent, ABT263, a dual Bcl-2 and Bcl-xL inhibitor, that can selectively kill senescent cells.

Dr Zhou explained that Bcl-xl is a well validated tumor target, but that the efficacy of ABT263 is hampered by dose-limiting thrombocytopenia. To reduce the toxicity of ABT263 his group converted it into DT2216, a Bcl-xL proteolysis-targeting chimera (PROTAC) that targets Bcl-xL to the von Hippel-Lindau (VHL) E3 ligase for degradation. DT 2216 is more potent against various Bcl-xL-dependent leukemia, lymphoma and cancer cells, but is considerably less toxic to platelets than ABT263 in vitro because VHL is poorly expressed in platelets.

They found that in vivo DT2216 effectively inhibits lymphoblastic leukemia and T cell lymphoma as a single agent, or in combination with other chemotherapeutic agents, without appreciable thrombocytopenia. These findings demonstrate the potential to use PROTAC to reduce on-target drug
toxicities and rescue the therapeutic potential of previously undruggable targets. Furthermore, DT2216 may be developed as a safe first-in-class antitumor agent targeting Bcl-xL.

Questions for Professor Daohong Zhou

Nadia Carlesso: That was an exciting presentation. How is it that DT2216 is specific; does it really bind only to Bcl-xL? This I did not catch.

Daohong Zhou: Yes. DT2216 is a specific Bcl-xL degrader. Although the ligand, ABT263, used to generate DT2216 binds BclXL and Bcl-2, DT2216 only degrades Bcl-xL. This is in part because DT2216 induces Bcl-xL degradation depending on lysine 87 ubiquitination and Bcl-2 does not have a lysine at the same site. This finding suggests that the accessibility of a lysine on the target protein is an important determinant for PROTAC (proteolysis targeting chimera) to degrade the target.

Hematopoietic stem cell heterogeneity is associated with myeloproliferative neoplasm

Dr Lihong Shi received her PhD degree in 2008 from the Institute of Zoology, Chinese Academy of Sciences, majoring in physiology. She gained her postdoc training at Professor Engel's lab at University of Michigan Medical School from 2008 to 2014. After that, she established her lab at the Institute of Hematology, CAMS/PUMC, in Tianjin, China, and currently her group focuses on the molecular regulatory mechanism of normal erythropoiesis and the pathogenesis of red cell diseases. She has since published more than 40 papers in scientific journals including Nature Medicine, Nature Communications, Nucleic Acids Research, and other prestigious journals.

Dr Shi discussed the heterogeneous nature of tissue stem cells and the implications of this heterogeneity for disease pathogenesis, which remain poorly understood. The JAK2V617F+ myeloproliferative neoplasms (MPNs), harboring the same JAK2 mutation in hematopoietic stem cells (HSCs), display diverse phenotypes, including polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). This scenario constitutes an instructive paradigm for analyzing the pathological consequences of stem cell heterogeneity. Single-cell gene expression profiling with parallel mutation detection demonstrated that the megakaryocyte (Mk)-primed HSC subpopulation expanded significantly in ET, and this expansion was driven primarily by JAK2 mutation. Her study provides evidence for a paradigm in which the specific pathogenic consequences of a disease mutation are at least in part determined by stem cell heterogeneity.

Questions for Li Hong Shi

Wang Min: What is the allele burden of homozygous and heterozygous JAK2V617F mutation in ET patients?

Lihong Shi: In ET patients, previous studies have demonstrated that JAK2V617F allele burden is usually low and is more frequently associated with heterozygosity. However,
Anti-IL-2 preserves graft vs leukemia activity while preventing graft vs host disease

Professor Defu Zeng obtained his MD from Fujian Medical University and postdoctoral training in transplantation immunology at Stanford University School of Medicine. Dr Zeng has been a full professor of Immunology/Hematopoietic Cell Transplantation at the Beckman Research Institute, City of Hope National Medical Center in California since 2013 and is internationally recognized in the preclinical field of graft-vs-host disease (GVHD) pathogenesis, prevention and therapy. Prof. Zeng is a pioneer in the field of induction of mixed chimerism as a curative therapy for hereditary hematological disorders and autoimmune diseases. He has more than 75 publications in high impact publications including in Science Translational Medicine, Nature Communications, JCI, JEM, PNAS, Blood and JI. Prof. Zeng had been a standing member of NIH study section of transplantation, tolerance, and tumor (TTT) from 2013 to 2017, and is now continued being an ad hoc reviewer for many NIH study sections.

Professor Zeng explained that donor T cells mediate both graft vs leukemia (GVL) and GVHD activity after allogeneic hematopoietic cell transplantation (AlloHCT). Development of methods that preserve GVL activity while preventing GVHD remains a long-sought after goal. His group reported that depletion of donor CD4⁺ T cells in transplants early after Allo-HCT augmented alloreactive CD8⁺ T cell energy, exhaustion and apoptosis in the GVHD target tissues but not in the lymphoid tissues, leading to prevention of GVHD while preserving GVL activity (Ni & Song et al. J Clin Invest. 2017;127 [5]:1960-1977). They hypothesized that the mechanisms may be through removal of IL-2 help from donor CD4⁺ T cells for donor CD8⁺ T cells, and administration of anti-IL-2 mAb can prevent acute GVHD. In the current studies, they found that administration of tolerogenic anti-IL-2 mAb (JES6.1) early after Allo-HCT in mice attenuated the severity of acute GVHD while preserving GVL activity that is dramatically stronger than observed with tacrolimus (TAC) treatment that blocks production of IL-2 in T cells. Anti-IL-2 treatment downregulates activation of IL-2-Stat5 pathway and reduces production of GM-CSF.

In GVHD target tissues, enhanced T cell PD-1 interaction with tissue- PD-L1 leads to reduced activation of AKT-mTOR pathway but increased expression of Eomes and Blimp-1, increased T cell energy/exhaustion, expansion of Foxp3⁺ Treg and Foxp3⁻ IL-10-producing Tr1 cells, and depletion of GM-CSF-producing Th1/Tc1 cells. In recipient lymphoid tissues, lack of donor T cell PD-1 interaction with host-tissue PD-L1 preserves donor PD-1⁻ TCF-1⁻Ly108⁻CD8⁺ T memory progenitors (Tmp) and functional effectors that have strong GVL activity. Anti-IL-2 and TAC treatment have qualitatively distinct effects on donor T cells in the lymphoid tissues, and CD8⁺ Tmp cells are enriched with anti-IL-2-treatment compared to TAC treatment. Thus, anti-IL-2 treatment early after HCT represents a novel approach for preserving GVL activity while preventing acute GVHD.

Questions for Professor Defu Zeng

Min Wang: You mentioned about the PD-L1 deficient mouse, and that PD-L1 deficiency is good for the prevention of GVHD.

Defu Zeng: No, excuse me. PD-L1 expressed by host tissue is required for prevention of GVHD via administration of tolerogenic anti-IL-2 (JES6.1).

Min Wang: Yes, but in the solid tumor usually PD-L1 is highly expressed, but in hematological malignancy patients usually PD-L1 cannot be detected in these patients, so in this kind of situation, how can you use this method for these patients in hematological malignancy?

Defu Zeng: You say that hematological cells do not upregulate PD-L1?

Min Wang: In the patients, for example leukemia patients, their leukemia cells have very low levels of PD-L1.

Defu Zeng: Yes, that’s good, it is good for GVL activity. So, donor CD8⁺ T cells can kill them.

Min Wang: How do you choose the anti-IL-2 clone for that?

Defu Zeng: You need to check the 3-dimensional structure of the anti-IL2/IL-2R complex. I tell you that Jeff Bluestone has developed an anti-human IL2 mAb that can form an anti-IL-2/IL-2R complex to promote IL-2 binding to IL-2Rα, and expansion of Treg cells that express high levels of IL-2Rα. The paper was published in Nature Medicine, by Meesiac et al. I am trying to have a collaboration with him for a clinical trial for preventing gut GVHD.

Nadia Carlesso: Does this clone prevent interaction of IL-2 with IL-2Rβ?
Defu Zeng: Yes, the tolerogenic anti-IL-2 mAb form an anti-IL-2/IL-2R complex that allows IL-2 binding to IL-2Ra but blocks IL-2 binding to IL-2Rβ.

Nadia Carlesso: I do not know about the size of the epitope, or about the 3D structure. Is this approach sensitive to those? So does the dose of the antibody matter? Can too much antibody be harmful? Did you titrate different antibody doses, and are there any differences in antibody doses?

Defu Zeng: We did not do titrations, we only tested the antibody according to the immediate paper I have cited.

Nadia Carlesso: Another question. Is the interaction of the IL-2 with their receptor changed because of this antibody? Do you think that the different effects are due to the different signaling doses? Is there a threshold for signaling that determines this difference? Or is it qualitative?

Defu Zeng: If you like, I can forward you the Bluestone paper and John Sprent Science paper. They show that the immune complex changes the 3-D shape, and somehow allow the IL-2 bind to the IL-2Ra but block IL-2-binding to IL-2Rβ. It is a qualitative change.

Tao Cheng: That’s a question we are interested in, because we do have the antibody program in the Institute, and still have an inventory of antibodies that need to be characterized. So please, do bring the idea to the clinical trial here.

Hematopoietic Stem and Progenitor Cells Chaired by Simón Méndez-Ferrer and Bing Liu

Notch ligands orchestrate the generation of hematopoietic stem cells in the embryo

Professor Anna Bigas holds a Bachelor of Science and a PhD in Biological Sciences from the University of Barcelona in Spain. She is currently a research group leader at IMIM in Barcelona and the scientific director of CIBERONC, a national cancer research collaborative network. Dr Bigas has a long-standing interest in hematopoietic stem cells, which started during research for her PhD thesis by characterizing human hematopoietic stem cells. As a postdoc at the Fred Hutchinson Cancer Research Center in Seattle her pioneering work identified a role of Notch in the regulating hematopoietic differentiation, a highly influential contribution to the field of hematopoiesis published in PNAS 1996, Mol Cell Biol 1998 and Blood 1999. Since starting her independent research group in Spain, she has sought to decipher the molecular mechanisms that regulate stem cell commitment, maintenance, differentiation and oncogenic transformation, mainly focused on the hematopoietic and intestinal system.

Prof. Bigas has demonstrated crucial roles for Notch and Wnt in the generation of hematopoietic stem cells in the mouse embryo, published in Development 2005; EMBO J 2008, 2020; JEM 2012, 2013, 2014; and Nature Communications, 2015. Her work helped to identify NFkB and β-catenin as a new therapeutic target for the treatment of T-ALL (in Cancer Cell 2010 and Leukemia 2016), thus contributing to the molecular understanding of this disease. In addition, Dr Bigas has actively participated in identifying novel functions for specific NFkB elements in other systems and published in PNAS 2004, 2007, 2009; Cell Reports 2012; Cancer Cell 2013; Leukemia 2018; Molecular Cell 2019, EMBO Reports 2020.

Professor Bigas pointed out that conserved Notch function is crucial for the specification and generation of hematopoietic stem cells (HSC) across evolution. By using genetically modified organisms, most of the elements that are functionally relevant have been identified, mainly in mouse and zebrafish. However, due to the disruptive nature of these studies, the physiological sequence of events that lead to and result from this specific Notch function is still uncertain. This is especially important in terms of reproducing this activity in vitro. The Notch system provides cells with a binary decision mechanism that takes place among neighboring cells. In vertebrate systems, several Notch ligands and receptors co-exist in the same cell and cells compete for activating the Notch receptor that will condition the fate of that cell. In the aorta-gonad-mesonephros (AGM) region, HSCs develop from hemogenic endothelial cells that reside in the ventral side of the dorsal aorta. Cells in the developing aortic endothelium co-express different types of Notch ligands and receptors. Several groups including theirs have helped to define the elements involved in this pathway, but several gaps remain for a complete understanding. In addition to the Notch1 receptor, Jagged1 and hes1, her group has recently shown how DI4 has an important structural function in the hematopoietic cluster composition of the aorta. Her group provided data on how blocking DI4 with a specific antibody impinges on the number of cells recruited into the cluster and the HSC activity. However, how to integrate all these different signals coming from DI4 and Jagged1 in the same or different hemogenic/hematopoietic cells is complex. Thanks to current reagents and methodologies, it is possible to access the transcriptome of cells expressing different ligands and receptors and distinguish those that activate Notch from the others. Their aim is to understand the Notch pathway at a cellular level in the hemogenic and hematopoietic cells of the AGM.

Questions for Anna Bigas

Simón Méndez-Ferrer: I was wondering about the role of Notch and DLL4 in regulating cluster size in relationship to the number of hematopoietic stem cells in the cluster? More broadly, whether the cluster size reflects the actual number of hematopoietic stem cells, or rather, the proliferation of hematopoietic stem cells, and whether this has any effect on self-renewal capacity?
Anna Bigas: Our results show a direct correlation between the larger size of the clusters after Dll4 treatment and an increase in the engraftment capacity of these cells in transplantation assays. At early embryonic time points of hematopoietic development (32-35 somites), this effect is more robust than in older embryos (38-40 somites). So, there may be some maturation effect on early HSCs that takes place in bigger clusters.

Engraftment of functional human hematopoietic stem cells in mice

The aim of Dr Claudia Waskow's studies is the identification of cell-autonomous and extrinsic factors governing maintenance and differentiation of hematopoietic stem cells and function of immune cells from mice and men over time. This includes the understanding of immune cell homeostasis, for example, their generation and turn-over during steady-state and under inflammatory and infectious conditions in young adults and during the aging process. The research of her laboratory, thus, focuses on uncovering basic mechanisms that regulate immune cell biology in the young and elderly, and this understanding may pioneer novel translational approaches. Xenotransplantation models allow for in-depth analysis of human hematopoietic stem cell (HSC) and immune cell function in vivo. Dr Waskow and colleagues generated novel mouse models supporting stable human HSC engraftment, which is a prerequisite for the continuous generation of all adult human blood cell types in mice. By introducing a loss-of-function Kit receptor into NSG mice they generated NOD/SCID Il2rg−/−KitW41/W41 (NSGW41) mice that combine an impaired endogenous HSC compartment with immunodeficiency that efficiently support stable engraftment of human HSCs in the long-term without the need for any conditioning therapy. As a consequence, multilineage engraftment including cells of the myeloid and erythroid lineages is highly improved in NSGW41 mice. Mechanistically, endogenous murine HSCs with a defective Kit receptor are largely replaced by human Kit-proficient donor HSCs. Further, “humanization” results in quantitative and qualitative changes of the mouse bone marrow microenvironment, suggesting that a mutual cross-talk between human HSCs and the mouse stem cell niche takes place. Using these novel recipient mice, the group aims at improving the outcome of xenotransplantation of islet cells by shaping the immune response in the surrogate humanized host.

Questions for Claudia Waskow

Simón Méndez-Ferrer: Thank you very much, Claudia, for this great talk. I can see that Hui Cheng has a question about whether human HSCs can use mouse stem cell factor from the mouse mesenchymal stem cells, and whether the improved engraftment is solely explained by SCF interaction between the mouse stromal cells and the human HSCs?

Claudia Waskow: Yes, great question. Experiments that have been conducted more than 20 years ago, actually suggested that human stem and progenitor cells (HSPCs) do not respond to stimulation with murine SCF, suggesting that our strategy should not have worked. It was always stated that the cross-reactivity was one way, thus, murine HSPCs respond to trigger with human SCF, but not the other way around. We have been repeating these assays with different outcomes. However, these are in vitro assays and our results strongly suggest that human HSCs can be triggered by murine SCF.

Simón Méndez-Ferrer: I also had a similar question, whether it is all related to SCF interaction or whether somehow the mouse stromal cells are instructed to support the human HSCs in a different way, so I wonder whether you have done for instance the co-transplantation of normal wild type stem mouse cells with the human cells to see whether they still have an advantage or not?

Claudia Waskow: Yes, we always wanted to do that, but unfortunately, we have not done this experiment yet. It’s a great experiment, we simply have not gotten around to doing it.

Simón Méndez-Ferrer: Have you seen whether in the other very beautiful model of lymphopoiesis and T cell studies which seems very useful, I was wondering whether you have observed some normalization of the T cell compartment or even over-production of T cells to some extent, and whether this is again correlated to remodeling of the niche and support of T cells in the thymus, or somehow you find expansion of stromal cells that might favor lymphopoiesis?

Claudia Waskow: We have not checked the stromal cells in the thymus. Do you mean “overproduction” as in having autoreactive T lymphocytes, or do you just mean expansion of the T cell pool?

Simón Méndez-Ferrer: Expansion of the T cell pool, whether it is the thymus size or you could have even excessive production of T cells at some point?

Claudia Waskow: It’s not an excessive production of T cells and we have no indication for autoreactivity. We use a BAC-transgene that mediates tissue-specific over expression of human IL-7 - a cytokine that is not cross reactive between human and mouse. In humanized NSGW41-hIL7-tg mice we see an enlarged thymus compared to humanized non-human-IL7 transgenic NSGW41 mice. However, it’s still not a normally sized thymus, but it is much bigger compared to the wild type found in non-humanized immune-deficient NSG mutant mice. Most importantly, the composition of
Multi-scale analysis of hematopoietic stem/progenitor cell function

Professor Bertie Göttgens graduated from Tübingen University in 1992 with a degree in biochemistry and received his DPhil in biological sciences from the University of Oxford in 1994. He then moved to the University of Cambridge Department of Hematology for postdoc training from 1994 to 2003 before becoming a Leukemia Research Fund Lecturer and then a University Lecturer (2003-2007). He was appointed Reader in Molecular Hematology in 2007 and since 2011 has been the University of Cambridge Professor of Molecular Hematology. In 2019 he was appointed Deputy Director of the Wellcome - MRC Cambridge Stem Cell Institute. Amongst other appointments he is an Associate Editor of Blood and a former president of the International Society of Experimental Hematology. He is a Fellow of the Academy of Medical Sciences and a member of EMBO.

Prof. Göttgens uses a combination of experimental and computational approaches to study how transcription factor networks control the function of blood stem cells and how mutations that perturb such networks cause leukemia. This integrated approach has resulted in the discovery of new combinatorial interactions between key blood stem cell regulators, as well as experimentally validated computational models for blood stem cells. Current research focuses on (i) single cell genomics of early blood development, (ii) modelling the transcriptional landscape of blood stem and progenitor cell differentiation, (iii) transcriptional consequences of leukemogenic mutations in leukemia stem/progenitor cells, and (iv) molecular characterization of human blood stem/progenitor cell populations used in cell and gene therapy protocols.

Questions for Bertie Göttgens

Bing Liu: A question from Anna: Can you estimate the number and percentage of the LT-HSC-1 and LT-HSC-2 from your model?

Bertie Göttgens: Yes, we can, but I am sorry I cannot remember the numbers. I think the HSC-1 is significantly larger than the HSC-2. I cannot remember the exact numbers, but I am very happy to share them. [Later, Professor Göttgens did check this, and the inferred estimates are that HSC1 is 5-times bigger than HSC2.]

Bing Liu: I have a question about your Hoxb8 cells. How about its function in hematopoietic development? Any reports?

Bertie Göttgens: I’m not sure there is much known, I’m not even sure you need to use hoxb8, probably a Hoxb factor is all you need in this model. We now have also the Hoxb5 knock-in mice, which are very good mice for tracking hematopoietic stem cells in the adult system. Hoxb4 has been used for a long time in the human and mouse embryonic stem cell field try to endow in vitro differentiated cells with hematopoietic stem cell activity, so I think there is probably overlap in what the various Hox proteins can activate.

Human hematopoietic stem and progenitor cell landscapes: Location matters

Dr Elisa Laurenti’s career in cell biology has focused on studying hematopoietic stem cells (HSCs) first using mouse models during her PhD with Prof. Andreas Trumpp in Lausanne, then with Dr John Dick in Toronto during her post-doctoral studies. There she established robust methods to study the function and molecular make-up of human HSCs. In 2014, she moved to the Cambridge Stem Cell Institute and established her own laboratory thanks to a Wellcome-Royal Society Sir Henry Dale Fellowship. Her research aims to understand how HSC function is regulated at all stages of human life to eventually improve treatment of blood diseases. More specifically, her laboratory currently focuses on i) understanding how the functional output of the human HSC pool changes over a human lifetime, at steady-state and under inflammatory conditions; ii) characterizing the molecular regulation of quiescence and its relevance to HSC expansion ex vivo and gene therapy.

In adults, most hematopoietic stem and progenitor cells (HSPCs) reside within the bone marrow (BM), giving rise to all mature blood cells. Yet at any given time, a small proportion of HSPCs circulate in peripheral blood (PB), and under severe stress and disease, the spleen can significantly contribute to blood production. However, the cellular, molecular and functional composition of circulating and extramedullary HSPC pools remains unexplored. Dr Laurenti discussed the single cell characterization of the adult human HSC pool found in spleen and non-mobilized PB, comparing and contrasting it to BM. Using matched and unmatched samples from deceased and living donors, her group profiled more than 50,000 single CD34+ HSPCs by scRNA-seq and 3900 single phenotypic hematopoietic stem cells / multipotent progenitors (HSC/MPPs) in functional assays. In BM, they found a topography of the hematopoietic hierarchy that supports continuous HSPC proliferation and blood production. In contrast, the...
cellular configuration in extramedullary tissues is positioned for lineage-primed demand-adapted hematopoiesis, including a molecularly distinct subset of HSC/MPPs not found in BM. PB HSC/MPPs sustain a unique differentiation potential and configuration in healthy conditions, but which become imbalanced with age and in hematological conditions. Overall, these data identify extramedullary cellular reservoirs for demand-adapted hematopoiesis and provide a framework of clinical relevance.

Questions for Elisa Laurenti

Bing Liu: How much expansion of spleen residence HSPC vs spleen colonization by HSPCs mobilized from the bone marrow goes on during abnormal extramedullary hematopoiesis, such as myelofibrosis?

Elisa Laurenti: In our data we certainly cannot distinguish, so in the spleen we cannot know what may have come from the bone marrow and what is a long resident of the spleen. There are no known markers for that, so we cannot do that. From the literature it seems that during stress the stress response will predominantly mobilize stem cells to go from the bone marrow to the spleen, and to specific niches there. It is thought that this applies also in disease.

Bing Liu: If there are no megakaryocytes generated in the spleen, but only erythroid cells why would the extramedullary unique cluster be defined as MEP rather than erythroid progenitors?

Elisa Laurenti: Yes, I think that is a good question, we call it MEP, but effectively if we look at the very early MEP there are low levels of expression of megakaryocytic genes in all organs. We call them MEP because they are at the very beginning of this trajectory towards erythroid and megakaryocytes but if you do look at the earliest MEP in the spleen and compare it to the bone marrow you do see that this low level expression megakaryocytic genes is even lower in the spleen than in the bone marrow, so I would say that from very early on megakaryocytic priming is decreased in the spleen.

Tissue Regeneration and Repair
Chaired by Terry Lappin and Ding Ai

Decoding the heterogeneous vascular niche in lung regeneration

Dr Bi-Sen Ding is a distinguished investigator at Sichuan University. He received his bachelor’s degree from Nanjing University and a PhD in Pharmacology from the University of Pennsylvania. During his studies at U Penn and postdoc research at Howard Hughes Medical Institute/Cornell University, he discovered that vascular endothelial cells produce paracrine factors which instruct liver and lung regeneration and repair, functionalizing a “vascular niche”. These findings were published in Cell, Nature, Blood and Circulation.

Dr Ding initiated his independent research as tenure track Assistant Professor at Cornell in 2013. Since then, his lab has demonstrated that the pro-regenerative cues from vascular endothelial cell can be subverted to promote fibrosis or tumorigenesis. Therapeutic targeting of this “maladaptive vascular niche” can enable clinical strategy to promote organ regeneration and to block fibrosis and tumorigenesis. His ongoing research has further demonstrated that perivascular hematopoietic and fibroblast cells regulate the vascular niche in a divergent manner, differentially promoting either regeneration or fibrosis/tumorigenesis in injured organs. These findings are published in Nature, Cancer Cell, Nature Cell Biology, Nature Medicine, Science Translational Medicine, and Developmental Cell.

The research goal of Dr Ding’s group is to coax the damaged organ to regenerate and bypass fibrosis. Upon injury, organs such as liver and lung undergo regeneration and sometimes scarring. Overwhelming scarring constantly causes irreversible fibrosis in injured organs at the expense of regeneration. They are focused on illustrating how blood and vascular cells form an instructive microenvironment (as a “hematopoietic-vascular niche”) to jointly control organ regeneration and fibrosis. Dr Bi-Sen’s group has shown that blood and vascular cells produce epithelia-actively factors in the damaged organs to regulate the fate of facultative stem cells. Unfortunately, this pro-regenerative signaling landscape of hematopoietic-vascular niche is frequently overturned in diseased organs, leading to fibrosis and sometimes tumorigenesis. Therefore, the group aims to decode the cellular and molecular mechanism involved in the dynamic crosstalk between hematopoietic-vascular niche, parenchymal stem/progenitor cells, and mesenchymal cells in organ repair. Building on these mechanistic studies, we seek to design regenerative therapy approach to stimulate fibrosis-free organ repair, especially by editing the hematopoietic-vascular niche to facilitate the engraftment of transplanted parenchymal stem cells.

Question for Bi-Sen Ding

Ding Ai: It has been reported that lung also has megakaryocytes. Have you identified any in your study and what is the change during the lung repair?

Bisen Ding: Yes, that is actually what we are very interested in. We had a paper about 5 years ago showing that platelets are recruited after lung injury, and they release SDF-1 being tracked with endothelial cells. But as you know
megakaryocytes are becoming more and more interesting, especially the megakaryocytes inside the lung. So, we are not quite sure if the megakaryocytes inside the lung are contributing here, but we believe they have a very important functional contributing here. They are pretty much a reservoir of different cytokines and chemokines. We think they also play a very diverging role here, pretty much like endothelial cells. They could even promote regeneration or cause fibrosis. Thank you.

**Developing cell therapies for vascular repair in diabetes**

Professor Reinhold Medina completed his medical training and obtained his MD from San Agustin University-Peru in 2000. He was awarded his PhD in Stem Cell Biology from Okayama University Medical School, Japan in 2006. He was then recruited to Queen's University Belfast, UK. After two consecutive postdoctoral fellowships from JDRF International and Fight for Sight in 2008 and 2010, he was appointed Lecturer in 2012, promoted to Senior Lecturer in 2017, and Professor in 2020. Reinhold has a keen interest in understanding basic science to explain disease. His work on human endothelial progenitor cell biology is internationally recognized. His research focusses on the development of a stem cell therapy for regeneration of blood vessels with important implications for diabetic vascular complications. His group works on vascular stem cell biology which could lead to meaningful new approaches for the treatment of diabetic vascular complications.

Professor Medina summarized major health problems worldwide caused by diabetes. Although there have been significant advances in hyperglycemia management, morbidity and mortality related to diabetic complications remain high. Diabetic complications include atherosclerosis, retinopathy, and nephropathy. Their pathogenesis is driven by hyperglycemia-induced damage of endothelial cells and pericytes leading to hypoxia, ischemia, and tissue dysfunction. Therefore, diabetes has been considered as a disease of blood vessels. A logical approach to avoid diabetic vascular complications is to promote vascular repair; however, endogenous vaso-reparative cells in diabetic patients are impaired in number and function. The Medina group has identified a subtype of endothelial progenitor cells known as endothelial colony forming cells (ECFCs) as an ideal candidate to develop cell therapies to revascularize ischemic tissues in diabetic patients. ECFCs are consistently isolated from umbilical cord blood, have a well-defined immunophenotype CD31+CD146+CD45-CD14-, and exhibit diploid normal karyotypes. ECFCs unequivocal endothelial phenotype was proven by transcriptomics. ECFCs possess single cell clonogenic capacity, and effectively form vascular networks in vitro and in vivo. Importantly, the capacity of ECFCs for vascular repair has been demonstrated in several preclinical animal models of tissue ischemia. Data from the Medina group show that ECFCs revascularize the murine ischemic retina and therefore hold potential for treating early stages of diabetic retinopathy.

**Questions for Reinhold Medina**

**Terry Lappin:** Is immunorejection of the injected cells a serious problem?

**Reinhold Medina:** In the retina we do not see immune rejection. It is thought that the retina is somehow special and is protected. But the cells do have low immunogenicity, so when we do the analysis we can show that they are immunogenetic, but much less so than immune cells. We are proposing an allogeneic therapy using cord blood and for that we will do HLA matching as for a cord blood transfusion, and match 4 of 6, or 6 of 8, depending on the HLAs and the protocol. Another approach is to use immunosuppression locally with corticoids. Finally, an exciting approach would be to use universal cells with knockdown of HLA1 and HLA2 and overexpression of CD47 (the do not eat me signal) which would not induce immunorejection.

**Terry Lappin:** What are the potential negative effects of endothelial cell therapy?

**Reinhold Medina:** Obviously you do not want the cells to cause any harm. In the case of the retina it is a question I am frequently asked because the current therapy for diabetic retinopathy is only for the later stages when you get pathological new vessels, that is, too many blood vessels which are leaky and cause hemorrhage into the region so ophthalmologists are injecting anti-BGF to stop blood vessel formation, but that is later stage. In the early stage you want to repair vascular hypoxia with endothelial cell therapy. It is important not to treat patients with tumors because the endothelial cells could improve the blood supply to the tumor.

**Peter:** So, after injection, did these ECFCs show differential capacity to differentiate to the artery, vein, or capillary?

**Reinhold Medina:** Thank you Peter, very interesting question. When we do transcriptomics and proteomics before injection it is interesting that the cells are immature so that shows that they have low expression of artery, vein, and capillary markers so when they receive early passage they express the markers for artery, vein, and capillary. When we inject them, we only have the tissues. Our cells seem to adopt the markers of the tissues. Our cells seem to adopt the markers of the tissues. So, we have done ischemic retinopathy and ischemic limb. If the ECFCs are in the vein they adopt vein markers, in the arteries they adopt artery markers and so on. But we have looked only at a handful of markers, no more than 2 or 3 markers. We believe they do, but we probably need more
evidence. Currently in the lab, in unpublished work we are coculturing the cells with differentiated cells, so we have ECFCs + microvascular cells, ECFCs + human coronary artery endothelial cells. I can tell you that by doing single cell transcriptomics, so far, the data show that they do seem to be plastic.

Anna Krasnodembskaya: Are you planning to test ECFCs in larger animal models more relevant to human disease?

Reinhold Medina: This is an interesting question, Anna. How do you choose your animal model? We want to ensure safety for humans, but the choice of the animal model depends on the regulatory authority, MHRA in the UK, EMA for Europe and the FDA for the United States. How you choose your model depends on the target. In our case of the retina most of the animal models are accepted at least by the MHRA and sometimes by the FDA. For wound healing you would probably need to choose the pig, but that depends on the regulatory body.

Revealing cellular heterogeneity, developmental trajectory, and novel subpopulations with immune functions in megakaryocytes

Dr Jiaxi Zhou is a principal investigator and associate director of the State Key Laboratory of Experimental Hematology, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College. He obtained his PhD from the Graduate School of the Chinese Academy of Sciences in 2004, and his postdoctoral training at the Stowers Institute for Medical Research and the University of Illinois at Urbana-Champaign from 2005-2010. He joined the SKLEH as a group leader at the end of 2010. Dr Zhou’s laboratory has a long-standing interest in the study of early hematopoietic differentiation, generation of megakaryocytes (MKs) and platelets from human pluripotent stem cells, as well as hematopoietic disorders modeling with hPSCs. His group has recently captured the dynamic transcriptomic landscape at the single-cell resolution. They also found that the MK subpopulation enriched with immune-associated genes is present at the various developmental stages and is generated along a unique trajectory from MK progenitors. Furthermore, they identified two surface markers, CD148 and CD48, for mature MKs with immune characteristics, allowing them to define a novel CD148+ CD48+ MK subpopulation with immune potency. At the functional level, these CD148+ CD48+ MKs can respond rapidly to immune stimuli both in vitro and in vivo, exhibit high-level expression of immune receptors and mediators, and might function as immune sensors and recruit immune cells to the peripheral blood to participate in immune regulation. Their findings reveal cellular heterogeneity and a novel immune subset of MKs and should greatly facilitate the understanding of the divergent functions of MKs.

Questions for Jiaxi Zhou

Anna Bigas: How early do MKs appear in the yolk sac (YS)? Are they CD45+? We find CD45 negative MK in the AGM at around embryonic day (D) 10.5.

Jiaxi Zhou: Actually, that is a very good question. I cannot really remember if it is exactly the same time, I think it is 10.5 maybe, actually, it is quite difficult to collect samples at early stage. In our study, we chose two time points, D10.5 or D11.5 to collect tissues and cells. As to the cells, I think they are not CD45 positive, if I remember correctly.

Ding Ai: What happens to the absolute number of megakaryocytes after E. coli infection; do they all increase or just the percentage of CD48hi cells?

Jiaxi Zhou: After infection, interestingly the platelets drop very quickly within 2 hours, while the number of megakaryocytes are not upregulated. At the very beginning they might drop a little bit, but in general they can maintain numbers similar with the beginning stage. It is very surprising to see the total number not changed. But the CD48 positive cells were increased. We speculate they must have some interesting functions.

Ding Ai: How do CD48+ MKs recruit neutrophils? Through adhesion molecules or chemokines?

Jiaxi Zhou: In our study, we just incubated MKs and neutrophils together, so there is not enough evidence to confirm the underlying interaction between them, so both adhesion molecules and chemokines are possible I think, we need further experiments to reveal it.

Mitochondrial transfer by mesenchymal stem cells as a strategy for lung repair

Dr Anna Krasnodembskaya holds a Reader (Associate Professor) post at the Wellcome-Wolfson Institute for Experimental Medicine, Queens University of Belfast, UK and leads a group of postdoctoral researchers, PhD, Masters’ and
undergraduate students. Her studies are focused on the development of mesenchymal stem cell-based therapies for acute respiratory distress syndrome and investigation of the mechanisms mediating the MSC effect. Ongoing work is investigating the role of mitochondrial dysfunction in the pathogenesis of ARDS and the ability of MSC-derived extracellular vesicles to alleviate it through transfer of healthy mitochondria and miRNAs.

Dr Krasnodembskaya earned her Master’s and then Doctorate in Biology at St. Petersburg State University, Russia. She was selected to be a member of Postdoctoral Fellowship Program and was appointed as Assistant Professor at the School of Biological Sciences. Her second postdoctoral training position was in Professor Michael Matthay’s laboratory at the University of California, San Francisco, before joining the Faculty at Queen’s University of Belfast in 2013. She was promoted to Reader in 2019. Anna has nearly 10 years’ experience in pre-clinical MSC research and her studies have informed the design of several clinical trials for MSC in ARDS and sepsis in USA, Canada and UK. Her publications in STEM CELLS, 2010, Thorax and AJP Lung, 2012 are recognized as seminal papers in the field (with more than 500 citations). Her publication in STEM CELLS in 2016 was acclaimed as the most impactful paper of the journal that year, and Dr Krasnodembskaya received the STEM CELLS Young Investigator Award in 2017. Her ongoing research is funded by the UK Medical Research Council and the Wellcome Trust.

Dr Krasnodembskaya pointed out that acute respiratory distress syndrome (ARDS) is a major cause of acute respiratory failure in critically ill patients requiring mechanical ventilation, has no effective treatment, and is associated with high mortality and morbidity.

Mitochondrial dysfunction and its potential mechanistic role in the evolution of lung diseases have become increasingly recognized as an important and translationally promising research field. Strategies aiming to protect mitochondria from injury or to enhance biogenesis are being actively explored as potential therapeutic opportunities. Mesenchymal stem/stromal cells (MSCs)-based therapy is considered a promising approach for ARDS because of their ability to target major aspects of ARDS pathophysiology. MSCs act through both cell contact-dependent regulation of the host cells and by secreting soluble factors and extracellular vesicles. Direct intercellular communication between MSCs and their target cells can occur through formation the tunneling nanotubules leading to direct exchange of cytoplasmic content (including organelles such as mitochondria and lysosomes) and resulting in the restoration of function of the host cells injured by the disease microenvironment. More recently, MSC-derived extracellular vesicles (EVs) have attracted significant attention as potent means of intercellular communication and it has been demonstrated by several groups (including her group) that MSC EVs also are able to carry mitochondria. Transfer of healthy MSC-derived mitochondria to epithelial cells has been associated with remarkable therapeutic efficacy in models of acute lung injury and asthma, and they have demonstrated that mitochondrial transfer resulted in metabolic reprogramming of primary human macrophages towards anti-inflammatory phenotype with enhanced phagocytic activity. MSC modulation of macrophages through mitochondrial transfer also was critical for immunomodulatory and antimicrobial effects of MSCs in the models of LPS and E. coli-induced acute lung injury. Furthermore, they have recently found that MSC EV-mediated mitochondrial transfer is a novel mechanism in promoting human distal lung epithelial repair. Her group’s ongoing work is investigating the role of mitochondrial dysfunction in the impairment of alveolar-capillary barrier in ARDS and the ability of mitochondrial transfer mediated by MSC-derived extracellular vesicles to alleviate these.

**Questions for Anna Krasnodembskaya**

**Dr Hui Cheng:** Interesting talk, and now we also want to link exosome and mitochondrial transfer in our study, we used the dendra2 mice. If we used the transwell assay we did not observe mitochondrial transfer from MSCs to hematopoietic cells, but if we used cell-cell contact or coculture we can see mitochondrial transfer. This result indicates that mitochondria cannot be transferred via exosome. Can you please give us some advice or thoughts? Also, did you see mitochondrial transfer from other cells to MSCs?

**Anna Krasnodembskaya:** Thank you very much, very good question. From our experience, actually we do see mitochondrial transfer in the transwell system and of course we have shown evidence that they are secreted through the FSL vesicle. In terms of advice, maybe think about the pore size that you used in your transfer system, maybe the pores are too small so that microvesicles cannot pass penetrate, maybe that is the reason that you cannot observe mitochondrial transfer with transwells. Do we see mito transfer from other cells to MSCs? I think, yes. We did some pilot experiments; we did not go into that in depth. But, in the pilot experiments when we incubated with macrophages, or we incubated with endothelial cells and A549 cells, there was a reciprocal transfer.

**DAY 2 KEYNOTE SPEECH**

**Professor Anthony Atala**

**Introduced by Professor Linzhao Cheng**

Next morning, Professor Linzhao Cheng introduced the second Keynote Speaker, Anthony Atala, the G. Link Professor
and Director of the Wake Forest Institute for Regenerative Medicine, who is also the Editor-in-Chief of STEM CELLS Translational Medicine.

Professor Atala has achieved many notable accolades for his pioneering work in regenerative medicine. He was elected to the Institute of Medicine of the National Academies of Sciences and is a recipient of the US Congress funded Christopher Columbus Foundation Award, bestowed on a living American who is currently working on a discovery that will significantly affect society; the Edison Science/Medical Award; the R&D Innovator of the Year Award; and the Smithsonian Ingenuity Award for Bioprinting Tissue and Organs. Professor Atala's work was listed twice as Time magazine's top 10 medical breakthroughs of the year and was ranked by the Project Management Institute as one of the top 10 most impactful biotech projects from the last 50 years.

Professor Atala was named by Scientific American as one of the world's most influential people in biotechnology, by U.S. News & World Report as one of the 14 Pioneers of Medical Progress in the 21st Century, by Life Sciences Intellectual Property Review as one of the 50 key influencers in the life sciences intellectual property arena, and by Nature Biotechnology as one of the top 10 translational researchers in the world.

In introducing Professor Atala, Professor Linzhao Cheng pointed out that he had not met Tony in person, but that they had interacted over the past 10 years, particularly when Tony took over the leadership of STEM CELLS Translational Medicine, the sister journal of STEM CELLS. Tony founded the Wake Forest Institute for Regenerative Medicine in 2006, probably one of the first in the U.S. and the world. Tony was trained as doctor. He is still a practicing surgeon, and he has made many contributions in organ and tissue repair and regeneration. Tony has written many books and many papers, and a recent search of Google Scholar showed that he has total citations of more than 35,000. In addition, Tony is an inventor; he has many U.S. and international patents. So, in many ways Tony is pluripotent!

"Today, Tony will give us his insight and focus in his lecture entitled: Regenerative medicine: current concepts and changing trends."

In his opening comments Professor Atala thanked Professor Tao Cheng and the organizers of IFSC 2020. In terms of STEM CELLS Translational Medicine, he paid tribute to the truly pioneering work by Dr Marty Murphy and Dr Ann Murphy, who were in attendance at the online lecture and who were instrumental in establishing the publishing field for stem cells with the very first journal in the world dedicated to stem cells, many decades ago before many people had even heard of the words “stem cells”.

Dr Atala said, “It is such a wonderful pleasure to have this collaborative work with this Meeting through the journal STEM CELLS Translational Medicine. It is a great pleasure to be present with colleagues and friends,” and spoke of his appreciation for everybody’s attendance at the Meeting.

Professor Atala gave a brief overview of the origins of regenerative medicine, which as a field of research has a history of more than 70 years. Then he asked, “Why have there been so few clinical advances?” Dr Atala explained that the previous limitations were challenges such as inability to expand appropriate cells in vitro, inadequate materials, and inadequate vascularity.

Then Professor Atala summarized his pioneering work in the past several decades in generating various tissues and organs. He further went into the details of the importance of the components for successful efforts in regenerative medicine and tissue engineering, which includes cells, scaffolds, and enabling technologies such as 3-D printing. Professor Atala then discussed in some detail vascularity in some lab-generated or regenerated organs such as kidney, and future approaches to improve the outcome of regenerative medicine.

Professor Atala explained that the severe shortage of donor organs for transplantation is worsening year-by-year due to the aging population but provides the impetus for alternative approaches. Regenerative medicine and tissue engineering apply the principles of cell transplantation, materials science, and bioengineering to construct biological substitutes that may restore and maintain normal function in diseased and injured tissues. Stem cells may offer a potentially limitless source of cells, and 3D bioprinting applications are being utilized for potential therapies.

In describing some of the 15 applications of these technologies developed in his laboratory that have been used clinically, Prof. Atala stressed the unpredictable and lengthy nature of the work required to expedite their translation to the clinic. Some of the successful clinical applications have taken decades to develop.

Drawing on his long career as a practicing surgeon and as a pioneer in regenerative medicine, Professor Atala’s presentation was acclaimed as a tour de force. His meticulous down-to-earth approach has reframed our thinking across traditional boundaries and provided an impetus to an area of medicine with broad practical implications.

Questions for Tony Atala

Linzhao Cheng: It is my understanding that the cell source you used is allogeneic. Are you concerned about immuno-rejection, even though you may just need a one-time implantation?

Tony Atala: Thank you, Linzhao, for that question. For everything I have shown you today, we were actually using the patient’s own cells. So, we go to the patient, we take a small biopsy from their organ, and we are using committed cells, so we are using committed progenitor cells that we expand, and we then put right back into the patient. So, we do not have to
worry about rejection. Of course, one thing is that it takes years to get that cell. That's why it takes us 20 years before we put a tissue into a patient. It literally takes us decades to bring these technologies from the bench to the bedside and to do so carefully and safely. As you know there are many, many, challenges along the road to make sure you can do so in a reliable manner.

Linzhao Cheng: Thank you, of course it takes a long time to the autologous, and then before you have bioprinting as well to make this individualized or personalized medicine, I guess is the way to go first, before we can talk about a scale-up, and then to benefit more patients.

Linzhao Cheng: Someone has sent a question: Is the same biomaterial used in the regeneration of different organs?

Tony Atala: Great question. Actually no, every organ has different materials. We may use some of the same materials, but a different mixture. What we do is to basically replicate the biochemical and structural properties of the tissue we are trying to replace. So if we are replacing something like a blood vessel it is going to require very different properties than a bone, as I mentioned in the talk, but the materials are different, we use a family of about 40 different biomaterials that we mix and match to recreate a structure that will fit those properties for that specific tissue, and again that also requires a lot of development. Of course, during the talk I showed you everything that worked, because we did over, and over, and over again, until it worked. But I could not begin to tell you that for every slide that worked I can show you 50 that did not work, until we got to the right formula. But once you get to the right formula, and you stick to it, then it will work time and again, but you have to do a lot of experimentation between the materials and the cells to make sure you get the right mix that will actually recreate your tissue time and again.

Terry Lappin: Tony, a very impressive talk! Can I ask you a very simple question? You are a surgeon; how do keep all the organs in an aseptic condition? Is that a major challenge?

Tony Atala: That's a great question, Terry, thank you. So, what we do basically is that in the moment that we create the organ we are using very sterile conditions. The cell cultures of course are entirely sterile, the scaffolds are entirely sterile, we use the incubators which are sterile, and then when we finally transfer the technology to a patient, and we have now 15 applications of our technologies to patients, in various stages, either stage 1 or stage 2 or even stage 3. Once you get it to the patient you need to use very specialized bioreactors, where we place the tissue inside the bioreactors which are entirely sealed, so that from the moment it leaves our facility to the time it gets to the surgeon it is basically in a sealed environment that cannot be opened, and it keeps things alive. Then it is opened steriley on the operating table, so that they can retrieve the structure and implant it surgically using aseptic techniques.

Linzhao Cheng: Tony, there is also a question asking what is the key challenge for FDA approval, assuming your biomaterial and the cells do need approval from the U.S. FDA?

Tony Atala: You know, the FDA have been great. I have to tell you that they have been great partners of ours. They are always willing to help; they definitely want to see these technologies advance. They do appreciate the fact that you are doing this slowly and carefully and making sure that you are not jumping the gun. Of course, everything we do goes through the FDA here in the U.S., you just need to pay attention, dot your i’s and cross your t’s, as you take these technologies to patients, but really we are all wanting the same thing. We all want safety for our patients and the FDA has done a remarkable job. We have great leadership right now at the FDA with Dr Marks and others, who have been really very positive about advancing these technologies to patients.

Linzhao Cheng: Thank you very much, Dr Atala. Because of time limits, we have to move on. Once again, we just want to thank you for sharing your time and telling us about you exciting work, as well as some of the new directions. Thank you very much.

Subsequently on Day 2, eleven speakers contributed to three scientific sessions.

**Regenerative Medicine**

**Chaired by Hideo Ema and Jinyong Wang**

**Hematopoietic stem cell expansion: Developmental pathway and clinical results**

John Wagner MD, Professor of Pediatrics, Director of the Institute of Cell, Gene and Immunotherapy and founding member of the Stem Cell Institute, is a clinical and translational investigator in the field of hematopoietic stem cell transplantation and immune cell therapies with more than 30 years of experience leading phase I/II/III clinical studies in the treatment of malignant disorders and rare genetic diseases. He is most well-known for the development of umbilical cord blood as a source of transplantable stem cells, performing the first transplant with cord blood in a child with leukemia in 1990. He subsequently developed the double umbilical cord blood platform that markedly increased the utilization of this stem cell source in the treatment of adults with hematological malignancies. His current research is focused on hematopoietic stem cell expansion using an aryl hydrocarbon receptor antagonist to speed engraftment after transplant, regulatory T cells to control graft-vs-host disease, thymic progenitors to enhance immune recovery. In addition, he pioneered the use of hematopoietic stem cell transplant as a vehicle for extracellular matrix repair in the
treatment of severe forms of epidermolysis bullosa and improved survival in children and adults undergoing transplant for Fanconi anemia.

Professor Wagner detailed the developmental pathway to the clinic, current results and next generation studies using expanded HSC, such as for manufacturing thymic progenitors to reduce the period of immune incompetence universally observed after allogeneic HSC transplantation. Hematopoietic stem cells (HSCs) are defined by their capacity of self-renewal and multipotency. It has previously been shown that nearly all repopulating capacity can be found in the CD34+CD90+ cell population. In recent years, the use of autologous and allogeneic HSCs has not only increased in the treatment of patients with refractory or relapsed lympho-hematopoietic malignancy but also in various non-malignant diseases, including the use of gene modified autologous HSC for selected inherited disorders. As limited quantities of HSCs are available from some donors, for example, umbilical cord blood (UCB), or after gene modification of autologous HSC, there has been considerable interest in the development of ex vivo expansion methods. High-throughput screening of thousands of molecules has identified several with HSC expansion potential; these include prostaglandin E2 (PGE2), UM171, a pyrimido-indole derivative, and StemRegenin 1 (SR1), an aryl hydrocarbon receptor (AHR) antagonist. The aim of his group's initial studies was to determine safety of the SR1 first in the setting of a double UCB transplant, with one unit unexpanded to minimize the risk of graft failure should SR1 culture lead to terminal differentiation of all HSC. Subsequent studies have focused on efficacy as well as evaluating the effect of using smaller, but better HLA matched UCB units as starting material, considering the 350-fold expansion potential of CD34+ cells with AHR antagonists.

Questions for Professor John Wagner

Hideo Ema: Thank you very much, that was a great talk. So far, everything looks so good. Let me ask you one question. It is 4 to 5 years since you published the paper. What about the long-term reconstitution?

John Wagner: Uniformly, we follow the patients through 5 years with engraftment studies and in every single case of those who surviving for 5 years, the patient remains fully grafted. In other words, there has been no loss of engraftment once you engraft, you always engraft. So that's important information. That data I did not show, but it is available for the original studies that I showed you, where the follow-up has been all for 5 years, so we should present at future meetings. It's a very good question, because you need to know that the reconstitution is permanent.

One thing you might also be referring is what happened to immune reconstitution? In our first studies, we saw that the time to T cell recovery was pretty good, but it took about 100 days before we saw good T cell reconstitution in adult patients. In recipients of non-myeloablative conditioning, we found that T cell reconstitution was slower because, you may remember, in the non-myeloablative conditioning some of the patients, specifically 4 of the 9 patients, received ATG as part of the conditioning. ATG clearly delays immune reconstitution, regardless of whatever else you are doing. So that's important to know. However, in the most recent study, all patients received full dose myeloablative conditioning without ATG and immune reconstitution will be discussed at a presentation coming up in February, where we will discuss T, B and NK cell reconstitution. However, in the most recent study, we looked at both phenotypic and functional studies of immune reconstitution in terms of immunization responses at Day 60. What is interesting is that we found that most the patients never had a CD4 count less than 200/μL, which is quite remarkable. They never seem to have severe lymphopenia or low absolute CD4 count, as is typically observed after transplant and most responded well to immunizations. Certainly, by choosing smaller units, doing the expansion culture, having better HLA matching in combination, I think has resulted in a much better immune reconstitution outcome.

Hideo Ema: There are people using platelet recovery as a marker of engraftment in the case of a former transplantation. What kind of marker can you use if you cannot use platelets or neutrophils as a marker? Any comment?

John Wagner: We are talking about chimerism, so we are looking at the graft ratio of the unique polymorphisms of the donor vs the patient, so what we did is that we sorted each cell populations, T cells, B cells, NK cells and myeloid cells after transplant, and we looked at their chimerism status. It was 100% donor cells based on SNPs and polymorphisms, so molecularly they are all donor. We did not rely just on platelet or neutrophil count recovery.

Hideo Ema: OK, thank you very much.

Use of human pluripotent stem cells to identify novel regulators of immune cell activity

Dan Kaufman is a Professor in Department of Medicine, Division of Regenerative Medicine and Director of the Cell Therapy program at the University of California-San Diego. Dr Kaufman does clinical work in hematology/BMT. His research focuses on the use of human pluripotent stem cells to study development of hematopoietic stem/progenitor cells, lymphocytes, and other mesodermal lineages. In particular, his studies have developed efficient means to produce natural killer cells from human ES cells and iPSCs suitable for new clinical applications to treat relapsed/refractory cancers - both hematologic malignancies and solid tumors. Current studies aim to translate use of these cells into clinical therapies and to
engineer these NK cells with receptors and other modifications to improve killing of cancer cells.

Professor Kaufman pointed to human pluripotent stem cells, which can provide a key resource for cellular immunotherapies. Studies from his group have demonstrated that natural killer (NK) cells can be efficiently derived from both human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), which can be engineered to better target refractory malignancies. hESCs and iPSCs serve as a platform to express chimeric antigen receptors and other modifications to enhance anti-tumor activity. Importantly, hESC/iPSC-derived NK cells can be expanded to clinical scale in current GMP compatible conditions. Since NK cells function as allogeneic cells, this strategy enables use of hESC/iPSC-derived NK cells as an “off-the-shelf” targeted cellular immunotherapy against refractory malignancies. In addition, his group has done a CRISPR/Cas9-mediated screen of tumor cell lines to identify novel regulators of NK cell-mediated activity. These studies demonstrate that deletion of a gene that regulates tumor cell production of exosomes can lead to enhanced NK cell-mediated killing, suggesting a novel strategy to improve anti-tumor cell-based therapies.

Questions for Professor Dan Kaufman

Jinyong Wang: Conventionally, CAR sequence elements are introduced into fully developed mature T cells or NK cells for immunotherapy. But in your strategy, you introduced these modifications at the stem cell stage. I am wondering if there are any discrepancies between these two strategies in terms of NK cell maturation and functionality?

Dan Kaufman: We do not see any difference in NK cell maturation between CAR-NK cells that are derived from the pluripotent stem cells compared to our non-CAR NK cells, or NK cells isolated from peripheral blood. Overall, there are more similarities than differences between cord blood-derived NK cells, peripheral blood NK cells and iPSC-derived NK cells. There are some differences in maturation, such as differences in KIA gene expression pattern. But we do not find that those differences impact the activity of iPSC-NK cells. Certainly, there are more comparisons that could be done, but we do not find any notable differences with these engineered iPSC-derived NK cells.

Jiwang Zhang: Beautiful study. Like T cells and CAR-T cells, do you see the long-time memory T cells exit that may last longer than NK cells?

Dan Kaufman: Right. The important differences, so these are being used as allogeneic cells, so as opposed to autologous CAR-T cells that aren't rejected you get much longer persistence. I do not know specifically the clinical data for the iPSC-derived NK cells, but other NK cells tend to survive on the order of a few weeks. The advantage is that for these clinical trials is that you can do repeat dosing since we can make hundreds or thousands of doses of these iPS-NK cells at a time, patients are getting 3 to 6 doses as part of these trials, and so we think about scenarios that actually provide more precise dosings of these cells rather than to just to give everything that you make up front. And so, it is a little bit different paradigm than doing the CAR-T cells which is sort of a one-off therapy. Of course, people are looking at allogeneic CAR-T cells as well, but you really get into the same issues, with immune rejection there, I think, as you get with allo NK Cells.

CAR-NK cells from engineered pluripotent stem cells: Off-the-shelf therapeutics for all patients

Dr Shi-Jiang (John) Lu received his BS degree from Wuhan University (1982), MS degree from Peking Union Medical College (1985), MPH degree from Columbia University (1988) and PhD degree from the University of Toronto (1992). Dr Lu is currently the President and CEO of HebeCell Corporation, focusing on the development and clinical translation of regenerative medicine and cell therapy technologies, especially iPSC-CAR-NK cells for the treatment of cancer, autoimmune, and viral infectious diseases. Before establishing HebeCell, he was the Senior Director of Research at Advanced Cell Technology/Ocata Therapeutics. Dr Lu is an expert in stem cell biology and regenerative medicine with 20 years’ experience. He has conducted translational research and novel therapeutic strategies utilizing human embryonic stem cells (hESC), induced pluripotent stem cells (iPSC) and their derivatives. The goal of his research is to generate hESC/iPSC-derived products for the treatment of human diseases. He also has extensive experience in process development and large-scale production of human PSC derivatives under defined conditions for clinical trials. Dr Lu is the inventor of more than 20 patents in the stem cell field. In an analysis of global stem cell patent landscape by Nature Biotechnology in 2014, Dr Lu’s patent applications and citations ranked No. 7 and No. 5, respectively. In addition to stem cell research, Dr Lu also has more than 10 years’ experience in cancer research.

In his talk, Dr Lu explained that despite the rapid advancement of immune therapies utilizing NK cells, the manufacture of high-quality NK cells at industrial scale remains a major technical challenge. Unlike donor sourced NK cells, human pluripotent stem cells (PSCs) offer an unlimited renewable source for NK cells. At HebeCell, they developed a novel 3D-bioreactor platform capable of producing high quality NK cells at industrial scale. More importantly, their 3D platform mimics the environment of secondary lymph tissues with continuous release of NK cells from these 3D spheres without stimulation of feeder cells, thus eliminating the process of exhaustive NK expansion that often compromises the potency of immune
cells. First, CAR-constructs were introduced into human iPSCs and stable and permanent CAR-iPSC clones were established. Secondly, CAR-iPSCs in 3D spheres were converted to hemogenic endothelial progenitors. By switching to conditions favoring sustained lymphopoiesis and NK cell development, large quantities of NK cells were released from spheres mimicking lymphoid organs. Up to 2.5 billion high purity iPS-NK cells (−95% CD56⁺) were harvested from one 500 mL bioreactor. These iPS-NK cells killed a variety of cancer cells including both blood and solid tumors such as pancreatic, ovarian and breast cancer cells in vitro, as well as multiple viral-infected cells. In summary, the new platform offers a viable alternative strategy for the manufacture of pure and potent NK cells at scales that meet the demand of unlimited doses of allogeneic off-the-shelf therapeutics for all patients.

Questions for Shi-Jiang (John) Lu

Hideo Ema: Thank you, Dr Lu. You showed a variety of bioreactors and you used that bioreactor for NK cells culture, but can we use that bioreactor for various kinds of cells, is that right?

John Lu: Yes, but for us the most important thing is to try to culture iPS cells in 3D conditions to create a microenvironment for NK cell differentiation. You can use them for other purposes, for example, we also collected a lot of blood progenitor cells including CD34⁺ cells from early differentiation. These progenitor cells can be further differentiated into other blood lineage cells, such as macrophage that are 80% to 90% purity and can be further expanded.

Hideo Ema: That is also the disposer, right?

John Lu: Yes, it is one-time use bioreactor.

Linzhao Cheng: Shi-Jiang, terrific talk, you have made a lot of progress in the past couple of years. In order to make a clinical product, I guess you need a GMP-certified iPS cell line or adapted iPS cell line. Can you tell us more about the starting material, the iPS cell, how do you derive that, and what is the certification in order to make a clinical product?

John Lu: This is a very nice question. There are a couple of companies that provide, at least they claim, GMP grade cell lines and ES cell lines. I believe Fate Therapeutics has their own GMP iPS cell lines; I believe they probably used Sendai virus technology to reprogram somatic cells into iPS cells. I think the Sendai virus GMP grade reprogramming kit is available through some commercial companies (CytoTune-iPS 2.0 Sendai Reprogramming Kit, Thermal Fisher). We are still discussing with a couple of companies, to try to get either the GMP grade reprogramming kit or get their cell lines. Our collaborator panCELLa in Toronto has GMP grade iPS cell lines. For our clinical program right now, I think we are going to use their cell lines.

Linzhao Cheng: The same question for Dan [Kaufman], can you tell us more about your starting material for the iPS?

Dan Kaufman: Yes, for the clinical product I am not involved in the manufacturing of those, so Fate Therapeutics has sourced or made those cells themselves. We are looking at some other projects that would do this GMP manufacturing ourselves. We do actually have a project using human ES cells that are GMP from WiCell which are readily available. There are also other commercially available GMP-suitable iPS cells but they are relatively limited. So, we have been discussing this issue. There are some companies being set that will make more GMP iPS cells as well. If people have good ideas for other sources of GMP iPS cells, I would also be interested to hear them, because they are in relatively short supply. What we do not know is whether it makes a difference and Shi-Jiang can chime in. Do you need one, or do you need more than one GMP iPS line to derive NK cell-based therapies? We have not found that again the KIR haplotype or the HLA haplotype makes a difference, and so it might not matter, but I think that is something that is still of interest.

John Lu: Good point, right now we are targeting two to three cell lines, just in case, because there are a lot of people who are asking the same question: what if you make a bad one? Will different iPS cell lines make any difference? Yes! But in our hands, we have tested almost ten cell lines; three ES cell lines, five or six iPS cell lines in our system and, basically, they are very similar. They are not identical, because each cell clone is different, but they are very similar. We have repeated more than 200 times for the 50 mL bioreactor, and 30 times for the 500 mL bioreactor, basically there is not too much difference. What I want to say is that if you adapt each iPS cell line into 3D conditions properly and make minor adjustment accordingly, most if not all iPS cell lines will work in our 3D-bioreactor system.

Molecular Mechanisms and Networks

Chaired by Jiwang Zhang and Jia Yu

Homotypic clustering of L1 and B1/Alu repeats compartmentalizes the 3D genome

Dr Xiaohua Shen is a Cheung Kong Scholar, associate professor in the School of Medicine and an associate investigator in the Center of Life Sciences at Tsinghua University. Her major research interest is to understand how the non-coding portions of the genome influence chromatin structure, gene expression, and stem cell fate in development. For some years, the Shen lab has rigorously investigated novel aspects of ncRNAs, genomic repeats, and RNA-binding proteins (RBPs) in the regulation of transcription, chromatin and genome organization. Her work facilitates the functional interference of ncRNA genes and brings about a paradigm shift in
our understanding of RNA and the noncoding genomes in transcription and chromatin regulation.

Dr Shen investigates novel functions of RNA-binding proteins in the regulation of transcription and chromatin states in pluripotent stem cells. Much of the developmental complexity of higher eukaryotes is thought to arise from gene regulation. RNA represents a hidden layer of regulatory information in complex organisms. Dr Shen's lab has rigorously investigated novel aspects of ncRNAs, genomic repeats, and RNA-binding proteins (RBPs) in the regulation of transcription, chromatin, and genome organization, in close collaboration with Dr Yujie Sun's lab at Peking University. Their work was recently published in *Cell Research*, “Homotypic clustering of L1 and B1/Alu repeats compartmentalizes the 3D genome”.

**Questions for Xiaohua Shen**

**Jia Yu**: How about the evolution of L1 and B1 sequence?

**Xiaohua Shen**: That's a very good question. So L1 and B1 are primate specific. In humans, mouse is B1, in humans it is Alu. They are closely related, so we think that in primates this should be a conserved role for these L1 and B1 repeats, at least in conserved in human and mouse. The main principles are the same in other species like fly and zebrafish, their respective repeat sequence may be different.

**Jia Yu**: There are many repeats sequence in the genome, so how to differentiate the important and weak repeats by functional assay?

**Xiaohua Shen**: This is just the beginning of the journey. We still know little about the protein regulators, so there is a long way to go. I think that we could develop some functional screening assays to identify additional regulators.

**Questions for Xiaohua Shen**

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**Questions for Feng Liu**

**Jiwang Zhang**: In the zebrafish with the Foxn1 knock out, which cells are reduced?

**Feng Liu**: Most hematopoietic cells are normal, the T cells are depleted, but the number of B cells is normal.

**Single-cell transcriptomes of blood cells and beyond**

Dr Ping Zhu has been an associate professor at Institute of Hematology and Blood Disease Hospital in Tianjin, China, since he finished his Ph.D. training at Professor Fuchou Tang's lab at Peking University. Zhu’s lab focuses on the study of cell identity determination, fate choice, and trajectory routes during differentiation in the hematopoietic system. The lab combines single-cell multi-omics techniques, at the genome, transcriptome, and epigenome levels, and computational analysis to dissect the molecular basis underlying these focused issues in both homeostasis and diseases. Another research interest is to develop computation tools to facilitate the biological insights mining for huge volumes of sequencing data.

Professor Zhu discussed single-cell transcriptome profiling approaches which offer new dimensions for determining the identities of blood cells in addition to regular immunophenotypes and retrospective functions. To facilitate the study of physiological and pathological hematopoiesis, his group is constructing construct the transcriptional atlas of blood cells in both human and mice by in-depth sequencing of immunophenotype-enriched hematopoietic stem/progenitor cells and mature cells from different lineages. Furthermore, by taking the atlas as references, they study hematopoiesis regeneration upon HSC transplantation in mice and bone marrow failure in humans. Notably, differentiation of HSC rather than expansion of HSC is observed.
immediately after transplantation. Professor Zhu’s group also study the cellular and molecular basis of immune attack leading to pancytopenia and pursue potential targets for therapy to improve hematopoiesis. In summary, the transcriptional atlases of human and mice blood cells are established and applied to the studies of HSC transplantation and bone marrow failure. Professor Zhu anticipates that these atlases of blood cells will serve as valuable resources for the in-depth study of hematopoiesis (http://scrna.sklehabc.com).

Questions for Ping Zhu

Jiwang Zhang: What’s the difference between the three HSC populations?

Ping Zhu: That is a good question. Actually, I do not show the data here, but we have published the differences in gene expression indicating their differentiation potential and lineage biases. For example, the third group of HSCs actually is biased towards lymphoid cells. There are differences between the three groups of HSCs.

Jiwang Zhang: What marker did you use to determine those three types of cells?

Ping Zhu: We used the single cell RNA seq technique to profile the gene expressions in single cells so actually we used thousands of genes to distinguish these HSCs.

Jiwang Zhang: Do you have some indicators for different stem cells?

Ping Zhu: Yes, of course, yes.

Jiwang Zhang: Were the changes in aplastic anemia patients associated with the splicing mutations?

Ping Zhu: We do not distinguish these patients by the splicing factor mutations, and we know that different mutations of splicing factors may have different effects. Actually, when we analyzed the aplastic anemia patients, we do see that the expression of splice effectors has changed, but we do not have the mutation data so I cannot answer this question at the moment.

CAMS-PUMC Webinar Series: Belt and Road Leaders
Chaired by Tsvee Lapidot, Hui Cheng, Lai Guan Ng

An instructive role for IL7RA in the development of human B-cells and B cell precursor leukemia

Professor Shai Izraeli is a physician scientist who focuses on translational laboratory-based investigations of pediatric hematological malignancies and on cancer predisposition syndromes. His vision is to promote innovation by integrating multidisciplinary basic, translational and clinical research to improve the care of children with cancer. He is particularly interested in studying developmental aspects of childhood leukemia. Over the last decade, his group has focused on leukemia predisposition syndromes, in particular Down Syndrome. They discovered a subtype of high-risk acute lymphoblastic leukemia (ALL) characterized by mutational activation of the JAK-STAT pathway (Lancet 2008; JEM 2011; Blood 2010, 2014; PNAS 2017). These discoveries have led to ongoing clinical trials of the children’s oncology group with JAK inhibitors for pediatric ALL. More recently they have focused on the metabolic adaptation of ALL to their microenvironment, especially the central nervous system (Nature Cancer 2020) and on the role of signaling and transcriptional regulation in normal and malignant hematopoiesis. These studies have been funded by multiple national and international grants and have involved multiple national and international collaborations in both basic and clinical research with scientists and clinicians in North America, Australia, Japan, and Europe.

Clinically, Professor Izraeli is the chair of the Division of Pediatric Hematology and Oncology in the Schneider Children’s Medical Center of Israel and is also affiliated to Tel Aviv University. They are leading the ALL trials in Israel as part of the European BFMAIEOP group. Internationally, he has been involved in leading positions in several hematology and cancer organizations. Most significantly, he has been a member of the Executive Board of the European Hematology Association (EHA), the previous chair of Biology and Diagnosis of the international BFM childhood leukemia group, the biology and clinical committees of the International Treatment of Children with Cancer (ITCC) and the co-chair of the biology committee of TAACL, USA.

Professor Izraeli explained that B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is preceded by a clinically silent pre-leukemia. Experimental models that authentically re-capitulate disease initiation and progression in human cells are lacking. Previously his group described activating mutations in the interleukin 7 receptor alpha (IL7RA) that are associated with the poor-prognosis Philadelphia-like (Ph-like) subtype of BCP-ALL. Whether IL7RA signaling has a role in the initiation of human BCP-ALL is unknown. IL7RA is essential for mouse B-cell development; however, human patients with truncating IL7RA germline mutations develop normal mature B-cell populations.

Professor Izraeli explored the consequences of aberrant IL7RA signaling activation in human hematopoietic progenitors on malignant B-cell development. Transplantation of human cord-blood hematopoietic progenitors transduced with activated mutant IL7RA into NOD/LtSz-scid IL2Rnull mice resulted in B-cell differentiation arrest with aberrant expression of CD34+ and persistence of pro-B cells that survive despite failing to achieve productive rearrangement of immunoglobulin V(D)J gene segments.
Activation of IL7RA signaling enhanced self-renewal and facilitated the development of a BCP-ALL in secondary transplanted mice. The development of leukemia was associated with spontaneous acquired deletions in CDKN2A/B and IKZF1, similar to what is observed in Ph-like BCP-ALL in humans. Single cell gene expression analysis suggested that pre-leukemic cells resided within a subpopulation of early B-cell precursors with CD34 + CD10highCD19low immunophenotype.

The development of a bona fide BCP-ALL from IL7RA transduced cells supports the hypothesis that aberrant activation of the IL7RA pathway in human B-cell lineage progenitors plays an instructive role by creating a pre-leukemic state which is vulnerable to transformation. These are the first demonstrations of a role for IL7RA in human B-cell differentiation and of a de novo Ph-like BCP-ALL development from normal human hematopoietic progenitors in vivo.

Questions for Shai Izraeli

Tsvee Lapidot: Thank you, Shai, for this elegant talk. Maybe I can start with the first question. What about treatment for Pre-B ALL both in Down Syndrome patients and in non-Down Syndrome patients since you see the signaling cascades which are different? Where do we stand currently with the potential therapy, and a sub-question to that, in particular, for the preleukemia as well?

Shai Izraeli: There was incredible excitement in talking about these findings, because of the JAK inhibitors, but what we discovered (PNAS 2017) is that the JAK2 mutated clones respond well to chemotherapy, and in relapse they are generally replaced by RAS mutated clones. This suggests that JAK inhibition will not be a good way to treat these leukemias.

The second phenomenon that we discovered, which may even be more interesting, is that low dose JAK inhibitors actually enhance the survival of B leukemia cells that carry activating mutations in JAK2. It was shown by Markus Müschen’s group that B cells are very sensitive to super-signaling, and if you block this signaling just a little bit it could enhance their survival. We discovered that this phenomenon also occurs genetically. Cells with JAK2 activating mutations acquire another genetic mutation in a gene called USP9X that balances the JAK2 enhanced signaling so that it will not be too much for their survival. So super JAK signaling can be also deleterious signaling for these cells. Really fascinating!

Tsvee Lapidot: Can you specify about the preleukemic cells, and if you can target those as well?

Shai Izraeli: Yes, that a very interesting question. In general, in pediatrics, because preleukemia is so common and leukemia is rare, it does not make sense to treat many children with preleukemia to prevent few leukemias. So maybe, the trick is to prevent the secondary mutation that leads to development of leukemia from pre-leukemia. There are now fascinating studies, that have just started to appear, that show that antibiotics that destroy the natural microbiome, facilitate the switch from preleukemia to leukemia. Importantly this happened in mouse models. So, it would be premature to conclude that treatment of children with antibiotics may increase their risk of leukemia. But maybe we can identify the microbial species that are important in protection from leukemia.

Liran Shlush: I do exactly the opposite of what Shai just said. He said it is hard to treat the preleukemic. I might claim that it’s the only way to do something in leukemia, is to treat the preleukemic, although they are very common. You just need to understand which one is going to become leukemic and which one is going to either disappear, or probably in pediatrics, disappear, Shai, not stay there for fifty years. Well, time will say who is right.

Shai Izraeli: In pediatrics, you are absolutely right. If we could have identified those that, either for certain would disappear, or for certain would develop leukemia, this would be great. But you know you have to take into account the practical issues. Can you imagine what is the significance of telling a mother that her child has a preleukemia and now we need to follow him for the next number of years? Especially, that the cure rate of childhood ALL is more than 90%. Is it impractical to follow all of the children who have preleukemia, just because of the risk that 1% of them will develop leukemia? So that makes the difference between adults and children. It is critical to diagnose the adult with preleukemia (clonal hematopoiesis), not because of the leukemia but because of they have an incredible role in atherosclerosis and other cardiac complications, but I’m entering into a minefield!

Liran Shlush: We will see.

Hui Cheng: I just wondered how you demonstrate the CDKN2a is the second hit, because you do not have functional study?

Shai Izraeli: We demonstrated that CDKN2A is a second hit by genomic analysis of preleukemia and leukemia cells. What we have been doing now is some functional studies.

Gene editing and high-throughput functional genomics

Professor Wensheng Wei received his bachelor’s degree in Biochemistry from Peking University, and PhD in Genetics from Michigan State University. After postdoctoral training and working as a research associate at Stanford University School of Medicine, Dr Wei became a principal investigator in the School of Life Sciences at Peking University in 2007. He is
now a professor of Biomedical Pioneering Innovation Center (BIOPIC), Beijing Advanced Innovation Center for Genomics (ICG), Peking-Tsinghua Center for Life Sciences (CLS), State Key Laboratory of Protein and Plant Gene Research, and School of Life Sciences at Peking University. The research of Wei group is mainly focused on the development of eukaryotic gene editing tools, with the emphasis on the high-throughput functional genomics and gene therapy. The combination of forward and reverse genetic means is employed, often in a high-throughput fashion, to develop a better understanding of the molecular mechanisms underlying human diseases, including cancer and infection.

Professor Wei discussed the development by his laboratory of a series of high-throughput screening (HTS) methods based on CRISPR/Cas9 system for the functional identification of protein-coding genes and long non-coding RNAs. His group has also re-designed an sgRNA scaffold that greatly boosts the efficiency and data quality for HTS. Their recent efforts include the identification of functional 3D-hubs that are essential for cell viability, the development of a new approach for mapping functional sites of protein of interest at single amino acid resolution, and a series of novel high throughput strategies derived from base editors. Besides these high-throughput strategies to facilitate the accurate and rapid identification of functional genomic elements in various settings, they have recently developed a novel programmable RNA editing strategy called LEAPER. Unlike conventional nucleic acid editing technology that requires simultaneous delivery of editing enzymes (such as Cas protein) and guide RNAs into cells, LEAPER enables precise and efficient RNA editing by recruiting endogenous cellular deaminases using engineered RNAs.

Questions for Professor Wensheng Wei

Lai Guan NG: Is it possible to employ these high throughput CRISPR technologies for screening primary cells, for example hematopoietic cells?

Wensheng Wei: It is definitely more challenging to conduct CRISPR screening for primary cells. To overcome this, that is the reason why we have developed two relevant technologies, (1) CRISPR-I Bar technology, technology, which provides us with a way to use high MOI [multiplicity of infection, which is used to define the ratio of viral or phage particles to cells in a system to infection targets] for library screening. Also, primary cells are sensitive to double strand break (DSB), that is the reason we also developed a technology called BARcode Base Editing-mediated gene KnockOut (BARBEKO) BARBEKO that could potentially facilitate screening of primary cells.

Lai Guan NG: For the Leveraging Endogenous ADAR for Programmable Editing on RNA (LEAPER) technique, is this a dose-dependent, or it is an “all or nothing” effect when this technology is employed for RNA editing?

Wensheng Wei: That's a very good question. It definitely has a dose effect. However, our RNA editing strategy is different from many others in that our technology can control the dose by delivering the ADAR-recruiting RNA (ArRNA) by oligo or viral backbone (eg, lenti or AAV system). It is heavily affected by the purity of the oligo, and also by how the synthesized oligo is protected for the delivery. So, this is very important and affects the efficiency of the LEAPER.

The aging of the blood system

During his postdoctoral research, Dr Liran Shlush examined genes commonly mutated in acute myeloid leukemia (AML), successfully identifying those “pre-leukemic” stem cells that go on to form cancerous cells. In more recent work, Dr Shlush used population-wide medical data available through a large repository of electronic health records (the Weizmann-Clalit project), along with deep sequencing techniques, to characterize the genes frequently mutated in the peripheral blood cells of individuals who later developed AML. Not only did this work form the basis of a model that accurately predicted AML-free survival, it also led to a model for identifying healthy individuals who are at risk for developing AML in the future. This research by Dr Shlush and his colleagues represents a paradigm shift thinking about AML, which has long been considered as an unpredictable and unpreventable disease. It will also generate an enthusiasm toward the possibility of AML prevention through early intervention in a high-risk population.

In his talk, Dr Shlush asserted that the ability to prevent diseases should be the number one goal of medical research. As humans age, their diseases become more complex (multifactorial) and, accordingly early diagnosis and prevention become strikingly complicated. The work of his group and of others provides evidence that the age of the blood system as reflected by the accumulation of mutations in hematopoietic stem and progenitor cells (HSPCs), also termed age-related clonal hematopoiesis (ARCH) is correlated with both leukemia and cardiovascular disease (CVD). As the aging of the blood system occurs many years before these diseases it becomes feasible to use parameters of the aging blood in a personalized matter to predict leukemia and CVD.

Questions for Liran Shlush

Shai Israeli: Interesting to see how this beautiful story evolved. You know in the clinic the most frequent double strand breaks occur in secondary leukemia after chemotherapy. So, what do you think about it, can it even be a possibility, that maybe this pathway can generate translocations?

Liran Shlush: Yes, this is a great question, Shai. First, this pathway can definitely create translocations. Actually, we recently noticed that the Bcr-Abl translocation in K562, is possibly a result of MMEJ. There is also some evidence that
it can cause copy number variation and we are looking into that. It also causes deletions outside of the exon, and we have some evidence that these are functionally important, so MMEJ is probably important in translocations and copy number variations. However, I must say that we do not see more CALR, ASXL1 and SRSF2-like deletions after chemotherapy, and the reason is that they are early events. So, if you with therapy create such deletions it can take another 20 years to get the disease. So probably if chemotherapy or radiotherapy creates double strand breaks, and they are repaired by MMEJ and that leads to copy number variation, then it will have a phenotype, because you need a major event to create the phenotype.

Lai Guan NG: one quick question. What kind of cell was used to recapitulate the deletion?

Liran Shlush: Thank you for the question. We used five different cell lines, all of them worked. We used CD34 cells, HSCs, we did it also in T cells, B cells, we saw MMEJ deletions in almost every cell type, but specifically for the SRSF2 deletions we got the best efficiency in HSCs.

**Identification of pancreatic islet resident progenitors**

Dr Yi Arial Zeng did her PhD at Simon Fraser University in Canada and postdoctoral work at Stanford University. She has been a principal investigator at the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences since 2010. Her research interest is to understand the regulatory mechanisms of adult stem cells in various tissues, and the interaction between stem cells and their niche. She has focused her efforts on the protein C receptor (Procr), which is a target of Wnt signaling, and has been established as a surface marker of adult stem cells in multiple tissues, including the mammary gland, the blood vessel endothelium, the hematopoietic system, and the ovarian epithelium. Her recent work demonstrates that Procr also marks the long-sought pancreatic islet stem cells.

The long-term goal of her research is to determine how the stem cell regulatory mechanisms have deviated in diseases, and to learn how to control the players in these machineries in vivo, as well as to provide ever-expanding stem cells in vitro for the purpose of regenerative medicine.

Dr Zeng explained that it has generally proven challenging to produce functional β cells in vitro. She described a novel Procr+ cell population in adult mouse pancreas through scRNAseq. The cells reside in islets, do not express differentiation markers, and feature epithelial-to-mesenchymal transition (EMT) characteristics. By genetic lineage tracing, Procr+ islet cells undergo clonal expansion and generate all four endocrine cell types during adult homeostasis. Sorted Procr+ cells, representing ~1% of islet cells, can robustly form islet-like organoids when cultured at clonal density. Exponential expansion can be maintained over long time periods by serial passaging, while differentiation can be induced at any time point in culture. Beta cells dominate in differentiated islet organoids, while α, δ and PP cells occur at lower frequencies. The organoids are glucose-responsive and insulin-secreting. Upon transplantation in diabetic mice, the organoids reverse disease. These findings demonstrate that the adult pancreatic islet contains a population of Procr+ endocrine progenitors.

**Questions for Dr Yi Zeng**

Hui Cheng: As EPCR is a receptor, not just a marker, do you know its function in the pancreatic islet progenitors, or did you knockout the Procr to check its phenotype?

Yi Zeng: Yes, we believe it also plays a functional role in progenitors. The simple experiment we have performed is in vitro in the organoid system, when we delete Procr, it completely abolishes colony formation, so it is functional important. And in vivo, it is harder to do because, although we have the Procr flox allele, it is hard to find a good Cre line to delete it. As I mentioned, this is a new cell type, all the known expression genes in the islet, for example insulin cre, are not expressed in the cell. Now we are generating better tools to delete the Procr specifically in stem cell.

Hui Cheng: I saw the gene RSPO1 is very highly expressed in the progenitor, so do you consider to use this cre?

Yi Zeng: Yes, exactly, we are generating this cre line.

Hui Cheng: Can EPCR also label human islet progenitors?

Yi Zeng: Yes, we are working on that. We do see Procr expression in human sections, but we are not sure this Procr equals that Procr yet.

Benjamin Dekel: That was a very interesting talk. Do you think it’s a professional stem cell? This would be a stem state, differentiated cells which are differentiating and undergoing EMT and then reversing into an epithelial cell that is, stem cell vs stem state?

Yi Zeng: I believe so, that the Procr cells are the professional stem cells, because what we analyzed was always in an uninjured or unstimulated situation, along normal development, normal homeostasis, about what they can give rise to by lineage tracing. Like in the long-term tracing you can see the contributions of those cells are not subtle, in normal homeostasis, so I believe they are professional stem cells that were neglected in the past.

Benjamin Dekel: Have you tried isolating the Procr cells, not the organoids, and transplanting them into the injured mouse model and seeing the effects of the stem cells themselves, after sorting? What did you see?
Yi Zeng: Just by sorting the cells the amount would be too little. We only got 1000 cells out of one mouse, so for example if we sorted from 10 mice then we would get 10,000 cells. Where we transplant is in the kidney capsule and we would wonder whether 10,000 cells transplanted there would be a good location for maturation. But we have not tried it, that were just my thought.

Lai Guan NG: Related to what Benjamin was asking, can you use the mouse to do deletions for instance a cre crossed with DTA, so that you delete this particular cell. Would it work?

Yi Zeng: Yes, it’s a good point, and we do have the cre DTA system. We conducted the experiment and the mouse eventually became diabetic, but we do think the experiment is dirty and cannot be conclusive, because, as I mentioned, Procr also marks stem cells in the endothelial cells, as well as other cells. Endothelial cells are a very important niche, so if we did it (ablate Procr+ cells by DTA), at the end we do not know whether it is because of the failure of islet stem cells or because of the endothelial cells, so we dare not jump to the conclusion yet. Therefore, we are making better genetic models, hopefully as specific as possible, to just affect the islet stem cells, not other cell types.

Lai Guan NG: From your RNAseq data, you showed that there are multiple subsets of this islet cell, so do you think under specific kinds of stress for the mouse, it will affect different types of the islet cell? Or is it just that if you introduce something like a high fat diet it will just affect certain particular subsets, or will it affect every single subset of the islet cells?

Yi Zeng: There is only one group of this cell (Procr+ islet stem/progenitor cells). We have not done the high fat diet experiments. We speculate that under high fat diet challenge, this population would likely be more active and give rise to more progeny cells.

Lai Guan NG: Thank you so much.

Epithelial cell therapies for kidney disease

Professor Benjamin Dekel received a BSc and MD degrees from the Technion and a PhD from the Weizmann Institute of Science, all with highest honors. He completed a Pediatric Residency at Sheba, Post-Doctoral Fellowship in stem cell biology at the Weizmann Institute and a Pediatric Nephrology Fellowship at the Schneider Medical Center. Prof. Dekel has served as a visiting Professor at the Institute of Stem Cell Biology, Stanford University, and is currently the Director of the Pediatric Stem Cell Research Institute and the Chief of the Division of Pediatric Nephrology at the Edmond and Lily Safra Children’s Hospital, Chaim Sheba Medical Center. He is Professor of Pediatrics, Human Genetics and Biochemistry and leads the Pediatric Research Center on Genes, Development and Environment at the Sackler School of Medicine, Tel Aviv University.

He is known internationally as one of the most innovative and highly recognized investigators in the field of human renal stem cell biology, cell therapy and renal regenerative medicine.

In the field of human kidney development and pediatric renal cancer, Prof. Dekel has pioneered the identification of human stem/progenitor cells and their use in tissue repair, regeneration and targeted cancer therapy. Some of his bench research identifying and targeting the renal cancer stem/initiating cell pool has been translated to clinical trials for relapsing kidney cancer in children. Moreover, Prof Dekel is moving towards the translation of his basic research in renal regenerative medicine, which applies novel modalities of cellular therapies with nephron progenitors, to bedside. This may allow kidney patients to delay the need for dialysis and shorten the ever-growing waiting list for a kidney transplant.

Prof. Dekel is an elected member of the American Society of Clinical Investigation (ASCI), the American Society of Pediatric Research (APS/SPR) and was recently elected as an inaugural member of the Israel Academy of Scientific Medicine. He has received multiple awards among which are the Youdim Prize for Excellence in Cancer Research and the Israel Medical Association Prize for Medical-Scientific Innovation.

Professor Dekel explained that the generation of nephrons in fetal life depends on the differentiation via a mesenchymal to epithelial transition (MET) of self-renewing, tissue-specific stem cells that give rise to different types of nephron epithelia and are confined to a specific anatomic niche in the nephrogenic cortex. Importantly, these cells may transform to generate oncogenic stem cells that drive pediatric renal cancer. His group has shown by genetic-lineage tracing that follows clonal evolution of single kidney cells that once nephrons are generated, cell replacement and cell growth is driven by fate-restricted uni-potential clonal expansions in varying kidney segments arguing against a multipotent adult stem cell model. They term this lineage-restricted progenitor characteristics. Lineage-restriction is similarly maintained during ex vivo human kidney growth and in murine kidney organoids grown in culture.

Finding ways to preserve and expand, ex vivo, the observed in vivo kidney-forming capacity inherent to both the fetal and adult kidneys is crucial for taking renal regenerative medicine forward. Some of the strategies that they are using to achieve this are sorting human embryonic nephron stem/progenitor cells, growing adult kidney spheroids/organoids or reprogramming differentiated kidney cells towards expandable renal precursors. Further, they have demonstrated beneficial functional effects of human nephron-forming progenitor cells in mouse models of acute and progressive kidney injury both by differentiation-dependent and paracrine mechanisms. The ability to promote kidney tissue restoration may be
relevant in patients with kidney disease and transform renal medicine.

Questions for Benjamin Dekel

Lai Guan NG: You showed in one your slides that when you injected a progenitor cell, I think, you were able to stop disease progression. How do you think that it works, because kidney requires different structures, does it mean that different progenitor cells can give rise to different structures in the kidneys, or how do you modulate that?

Benjamin Dekel: Great question. First of all, I do think that there is not a single multipotent stem cell in the adult kidney that gives rise to all nephron epithelia. This is in sharp contrast to the fetal kidney. Within the adult nephron there are facultative mature nephron epithelial cells that act as lineage restricted progenitors in each segment of the kidney. We therefore need to use a mixture of heterogeneous progenitors for generating different segments of the nephron following transplantation. 3D kidney spheroids that we grow in the lab harbor the precursors for the different kidney segments. The purposed mechanism of action of 3D spheroids after transplantation is as follows: first, they can replace tubular cells along the nephrons. Second, they open up to generate tubular structures de novo in diseased kidneys. These tubular epithelial structures long term engraft and generate anti-fibrotic and anti-inflammatory paracrine effects. Third, the tubular structures can potentially hook up to the host tubules thereby increasing nephron mass. So all together the mechanism of repair is multifactorial in its nature. I want to emphasize that we see beneficial effects after repeated cell therapy. Treatment with kidney spheroids is not magic, we need to engraft cells repeatedly so as to generate beneficial effects. We are treating a CKD kidney, it harbors a lot of fibrosis, and it is very diseased, so we anticipate the need for cell therapy every couple of months. In the mouse model cells were given every 3 weeks, and only after 3 cycles we noticed a positive effect at the biochemical/GFR level.

Lai Guan NG: Do you think that is because the cells somehow go to places to do something, or they secrete something to make it better. How does it work? Can you speculate?

Benjamin Dekel: We recently published this in Cell Reports. When you derive diseased cells from a diseased kidney you can see that following expansion and generation of 3D at the kidney spheroids the cells are rejuvenated. We see it by gene expression analysis, for instance looking at improvement of fatty acid oxidation pathways in the cells and many other processes. So, by growing 3D kidney spheroids you actually revitalize sick nephron epithelia that reside in the diseased kidney. Importantly, when you put them back into the kidney, they engraft long-term and secrete anti-fibrotic and anti-inflammatory molecules generating an anti-fibrotic effect, in addition to cell replacement. Very recently, when we transplanted a combination of EPCs and MSCs into kidneys, these cells generated vessels that connected to host vessels. So, it’s probable that nephron epithelia can also connect after falling. It could be that only the generation of vessels in diseased kidney by vessel forming cells (EPCs/MSCs), could improve renal function.

Tao Cheng: Very nice talk. I noticed you used a lentivirus to deliver OCT4 to make more kidney progenitors. But how can we envision the possible therapeutic implication of over-expressing OCT4? This might increase the chances of cancer development.

Benjamin Dekel: We did the OCT4 experiments to induce and expand clonal progenitors from the adult kidney. Our goal is to upscale our cultures in a short time frame and get billions of cells. and for that OCT4 was indeed efficient. Clinically, you would need a mechanism of taking out OCT4 prior to transplantation, and we tried many different ways, including CRISPR-Cas9, Cre-loxp systems, to eliminate expression of OCT4. However, these methods did not work very efficiently, and we could not get OCT4 totally eliminated cultures. Ideally, we would need a very efficient genetic system to clear out OCT4 before we transplant the cells. Thank you.

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