2018 BC Regenerative Medicine Research Symposium

Pharmaceutical Sciences Building
University of British Columbia

May 9th 2018

Vancouver, British Columbia

@BCRegMed

Contents:

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Agenda for the Day

8:15am-9:00am	**Registration and Poster Mounting**		
9:00am-9:30am	**Welcome and Introductions**		
	Fabio Rossi (BCREGMED) Cate Murray (Stem Cell Network) Andrew Booth (STEMCELL Technologies) Gordon McCauley (CDRD) Mike Kallos (Alberta RegMed)		
9:30am-10:40am	**Theme 1: Cellular Therapies (Chair: Francis Lynn)**		
	25 mins	Megan Levings	Engineered tolerance
	20 mins	Kevin Gregory-Evans	Cell therapy and the eye
	12 mins	Gursev Anmole	Characterization of HIV-1 specific t-cell receptors in individuals that show enhanced control of infection
10:40am-10:55am	**Break**		
10:55am-12:05pm	**Theme 2: Endogenous Repair (Chair: Paul Cassar)**		
	25 mins	Wolfram Tetzlaff	Is remyelination a target to repair the injured spinal cord?
	20 mins	Leigh Anne Swayne	Understanding endogenous plasticity mechanisms in the brain
	12 mins	Philipp Kramer	Differentiation of human pluripotent stem cells into small intestinal organoids for regenerative applications and tissue repair
12:05pm –12:30pm	**Selected Rapid Fire Talks** Xiaxoue Xu (PDF, Swayne Lab, UVic) – Poster #35 Sanam Shafaattalab (PhD Student, Tibbits Lab, SFU) – Poster #5 Riya Sharma (Research Associate, STEMCELL Technologies) – Poster #22		

	Diana Canals Hernaez (PhD Student, McNagny Lab, UBC) – Poster #1 Avinash Thakur (PDF, Hoodless Lab, BC Cancer) – Poster #48 Andrew Hagner (PDF, Zandstra Lab, UBC) – Poster #46		
12:30pm-1:35pm	**Lunch & Poster viewing**		
1:35pm-2:45pm	**Theme 3: Novel Technologies (Chair: Eric Jervis)**		
	25 mins	Cole Deforest	User-programmable hydrogel biomaterials to probe and direct 4D stem cell fate
	20 mins	Mark Ungrin	The process of science - Using technology to make research more efficient
	12 mins	Steve Booth	Profiling the small airway microenvironment in chronic obstructive pulmonary disease: New technologies bring new opportunities
2:45pm-3:00pm	**Coffee break**		
3:00pm-3:55pm	**Theme 4: Current Commercialization in BC (Chair: James Jaquith)**		
	15 mins	Lee Buckler	Lessons from a CEO on the frontier: Building regenerative medicine in B.C.
	15 mins	Adam Hirst	Cell quality considerations for human pluripotent stem cell culture
	15 mins	Richard Liggins	Preventing hypoglycemia in insulin dependent diabetes: Targeting the somatostatin receptor 2
3:55pm-4:20pm	**Selected Rapid Fire Talks** Elizabeth Bulaeva (PhD Student, Eaves Lab, BC Cancer) – Poster # 14 Chloe Christensen (PhD Student, Choy Lab, UVic) – Poster #21 Roza Valez Ghaemi (PhD Student, Yadav Lab, UBC) – Poster #39 Matthew Langley (MSc Student, Zandstra Lab, UBC) – Poster #42 Himanshu Kaul (PDF, Zandstra Lab, U of Toronto) – Poster #38		
4:20pm-4:30pm	**Closing Remarks (Fabio Rossi)**		
4:30pm-5:50pm	**Networking and Rambunctious Poster Judging Session** **Complementary Beer/Wine/Appetizers**		
5:50pm-6:05pm	**Poster/Rapid Fire Prize Announcements (Allen Eaves/Fabio Rossi)**		

Speaker Affiliations

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Cate Murray Executive Director, Stem Cell Network

Andrew Booth Chief Commercial Officer, STEMCELL Technologies

Mike Kallos Professor, Department of Chemical and Petroleum Engineering, University of Calgary; Professor, McCaig Institute for Bone and Joint Health; Associate Director, Pharmaceutical Production Research Facility (PPRF); Associate Director of the Center for Bioengineering Research and Education (CBRE); Alberta RegMed

Gordon McCauley President & CEO, The Centre for Drug Research & Development (CDRD)

Presenters/Session Chairs:

Theme 1: Cellular Therapies

Francis Lynn Associate Professor, Departments of Surgery, UBC; Investigator, BC Children's Hospital Research Institute **(Chair)**

Megan Levings Department of Surgery, UBC; Investigator, BC Children's Hospital Research Institute

Kevin Gregory-Evans Professor, Department of Ophthalmology, UBC; Eye Care Centre

Gursev Anmole PhD Candidate, Brockman Lab, Department of Molecular Biology and Biochemistry, SFU

Theme 2: Endogenous Repair

Paul Cassar Associate Scientist, Project Search and Evaluation, The Centre for Drug Research and Development (CDRD) **(Chair)**

Wolfram Tetzlaff Director, International Collaboration on Repair Discoveries (ICORD); Professor, Departments of Zoology and Surgery, UBC

Leigh Anne Swayne Associate Professor, Division of Medical Sciences, UVic

Philipp Kramer Scientist, Research & Development, STEMCELL Technologies

Theme 3: Novel Technologies

Eric Jervis Principal Scientist, STEMCELL Technologies **(Chair)**

Cole Deforest Assistant Professor of Chemical Engineering, Core Member, Institute for Stem Cell & Regenerative Medicine, Adjunct Assistant Professor, Department of Bioengineering, University of Washington

Mark Ungrin Associate Professor, Department of Comparative Biology and Experimental Medicine, University of Calgary; Alberta RegMed

Steve Booth PhD Student, Hackett Lab, Centre for Heart Lung Innovation, UBC

Theme 4: Current Commercialization in BC

James Jaquith Head of Medicinal Chemistry, Centre for Drug Research and Development (CDRD) **(Chair)**

Lee Buckler President & Chief Executive Officer, RepliCel Life Sciences Inc.

Adam Hirst Scientist, Rearch & Development, STEMCELL Technologies

Richard Liggins Chief Scientific Officer, Zucara Therapeutics Inc.

Rapid Fire Presenters:

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Himanshu Kaul	Post-Doctoral Fellow, U of Toronto
Riya Sharma	Research Associate, STEMCELL Technologies

Registered Attendees

The names, e-mail addresses, and other contact information contained in this booklet are private and confidential information solely for the use of the Symposium participants for communications with one another, related to the purposes of the Symposium. The use of the contact information in this package for the purpose of commercial promotion of a product or service is strictly prohibited.

The names of those attendees who did not consent to have their contact information made publically available have been removed from this list.

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Podocalyxin is a therapeutic target in carcinoma

Diana Canals Hernaez[1,2], Michael R. Hughes[1,2], Ismael Samudio[3], Calvin D. Roskelley[4] and Kelly M. McNagny[1,2]

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Podocalyxin is a CD34-related sialomucin highly expressed on tumor cells in a sub-set of breast carcinomas, where it is associated with metastasis and poor prognosis. Deletion of podocalyxin in MDA-MB-231, a highly aggressive human breast cancer cell line, attenuates both the growth of primary tumors and formation of distant metastases. In addition to podocalyxin expression in breast carcinoma, podocalyxin cell-surface expression in tumor cells is associated with the most aggressive carcinomas of the colon, prostate, bladder, pancreas and ovary. As in breast carcinoma, podocalyxin tumor expression correlates with poor outcomes and, in colon cancer, identifies patients that would benefit the most from adjuvant chemotherapy. These findings indicate that podocalyxin is a potential therapeutic target for several human carcinomas.

We developed two novel monoclonal antibodies (PODO83 and PODO447) against tumor-expressed human podocalyxin. Systemic treatment with PODO83 inhibits both primary tumor development and metastatic progression in mice with an established tumor burden. PODO447 recognizes a unique, highly tumor-specific glycosylation epitope, making it an exciting candidate for targeted therapeutics. We took two different approaches to make PODO447 a therapeutic antibody: 1) Chimeric Antigen Receptor T cells (PODO447 CART) and 2) Antibody Drug Conjugates (PODO447 ADC). PODO447 CARTs have potent cytotoxic *in vitro* activity on glioma and pancreatic cells. PODO447 ADC can successfully be internalized and have potent cytotoxic activity in both ovarian and pancreatic cells *in vitro*. Moreover, systemic treatment with PODO447 ADC can eliminate an established tumor in an *in vivo* pancreatic xenograft model. These results confirm that podocalyxin is an ideal target for immunotherapy in carcinoma.

#2

Murine sensitization with house-dust mite extract alters the immune response towards H1N1

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Background:
The 2009 H1N1 pandemic observed a large percentage of hospitalizations attributed to the asthmatic population, but the question remains unclear as to what biological mechanism led to this.

It is widely accepted that asthma induces a type 2 inflammatory response in the lungs, characterized by the overproduction of IL-4, IL-5, and IL-13. Previous research has identified IL-4 as a potent inhibitor of the anti-viral type-1 response through the inhibition of IFN-g. We hypothesized that a predetermined type-2 response through allergic sensitization of mouse airways may alter the immune response towards viral infections, thus resulting in greater morbidity.

Methods:
Using a previously validated model of allergic asthma, 6-8 week old male BALB/c mice were intranasally exposed to house dust mite extract (HDM) or phosphate-buffered saline (PBS). Intranasal instillations were performed 5 days a week for 2 weeks, followed by a single intranasal inoculation of pH1N1 (A/California/04/2009 - 50uL at $10^{6.4}$ EID_{50}/mL). HDM or PBS instillation continued for another 5 days, and mice were sacrificed on days 0, 1, 2, 5, and 8 post-viral infection (pi). IL-4 and IFN-g levels were measured in bronchoalveolar lavage (BAL).

Results:
All mice experienced significant weight loss upon exposure to pH1N1. PBS-exposed mice were able to stabilize their weight, while the HDM-sensitized mice continued to lose significant weight by day 8 pi (PBS+pH1N1: 15.86% ± 1.2%, HDM+pH1N1: 22.80%± 1.9%, $p<0.0001$). The BAL cytokine profile revealed that HDM-sensitized mice displayed significantly lower IFN-g levels on day 8 pi (PBS+pH1N1:1.47± 0.77 ng/mL, HDM+pH1N1: 0.23± 0.16 ng/mL, $p<0.01$), and significantly higher levels of IL-4 initially during the infection (PBS+pH1N1: 0.16±0.04 pg/ml, HDM+pH1N1: 7.08± 3.1 pg/mL, $p<0.0001$).

Conclusion:
Allergic sensitization using HDM results in greater morbidity when mice are infected with pH1N1.

This may be due to a predetermined type-2 response causing an overproduction of IL-4 early during the infection and a dampened IFN-g production on day 8 pi.

#5

Determination of the possible role of cardiac troponin I mutation in sudden cardiac death in infants (SUDI) using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs)

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Sudden unexpected death of an infant is devastating for families, especially when the cause of death is not identified despite rigorous autopsy and toxicology screening. We recently completed sequencing >70 genes from 191 such children, all of whom died within the first 20 months of age. We identified 10 infants who carried an identical mutation not identified previously. The mutation (R37C *TNNI1*) is in the fetal paralog of troponin I (TnI), a gene thought to be expressed in the heart only up to the first 1-2 years of life. We investigated the role of this *TNNI1* mutation in the death of "autopsy negative" infants due to its arrhythmogenicity.

We investigated the biophysical properties of R37C *TNNI1* mutation in reconstituted human thin filaments (RTF). Fluorometry was used to measure changes in RTF Ca^{2+} sensitivity. RTF with the TnI mutant R37C *TNNI1* have altered Ca^{2+}-binding properties, suggesting that changes in Ca^{2+} kinetics may be one of the underlying mechanisms of arrhythmogenicity in these infants. Furthermore, we generated R37C$^{+/-}$ *TNNI1* mutants in hiPSC-CMs using CRIPSR-Cas9, and simultaneously monitored voltage (V_m) and calcium (Ca^{2+}) transients through optical mapping. We observed normal intrinsic beating patterns under control conditions in R37C$^{+/-}$ *TNNI1* hiPSC-CMs but in the presence of 500 nM isoproterenol irregular V_m and Ca^{2+} transients were developed. In addition, R37C$^{+/-}$ *TNNI1* hiPSC-CMs did not show any indication of restitution with increased stimulation frequency and exhibited alternans at 75 bpm and higher. The WT hiPSC-CMs did not exhibit any sign of arrhythmogenicity even at stimulation frequencies of 120 bpm.

This study has the potential to provide critically needed physiologic and mechanistic bases to implicate this gene (and this mutation) in pathogenesis of sudden cardiac death in infants.

#6

SMAD4 mediated TGFb/BMP signalling in PDGFRa+ tissue-resident mesenchymal progenitors is required for maintaining murine skeletal homeostasis

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Mesenchymal stem and progenitor cells are found in most adult tissues, including bone, and contribute to tissue maintenance and regeneration. The Rossi and Underhill laboratories have identified two populations of tissue-resident mesenchymal cells in bone; EMC+ cells dispersed along the bone surface represent 'stem-like' mesenchymal cells that are responsive to injury, and PDGFRa+ cells found throughout bone encompass cells from committed osteogenic progenitors to mature osteocytes. We hypothesized that EMC+ and PDGFRa+ cells and the TGFb/BMP signalling pathway, known to have roles in bone homeostasis, play an important role in murine skeletal maintenance. Using endogenously regulated inducible EMC-CreERT2 and PDGFRa-CreERT2 strains, we generated mesenchymal cell-specific SMAD4 knockouts in which EMC+ and PDGFRa+ cells respectively lack the transcription factor SMAD4, a common mediator in TGFb/BMP signalling. Deletion of SMAD4 in EMC+ cells had no significant impact on bone homeostasis, however, deletion of SMAD4 in PDGFRa+ cells resulted in an overt skeletal phenotype arising within two weeks after tamoxifen-induced gene deletion. PDGFRaCreERT2/Smad4$^{Flox/Flox}$ mice undergo extreme skeletal remodelling, including increased osteoclast activity at the bone surface. Using RNAseq, histology, and gene expression analysis we reveal a dysregulation of osteoblast and osteoclast activity in PDGFRaCreERT2/Smad4$^{Flox/Flox}$ mice. While we are continuing to characterize the cellular and molecular mechanisms responsible for these changes, our work suggests that a tonic signal transduced by SMAD4 in osteogenic cells is required for bone homeostasis. Identifying cellular and molecular targets required for maintaining bone may provide new therapeutic targets for those suffering from debilitating bone disorders such as osteoporosis.

#7

Differentiation of human embryonic stem cells to insulin producing beta cells

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Diabetes is a disease characterized by chronically elevated blood glucose levels that is a direct result of beta cell dysfunction and insulin deficiency. Current treatments include the use of insulin injections, donor islet transplantation or a combination of both. While insulin injections can provide some relief, patients are also at risk of life threatening hypoglycemia due to incorrect dosing of insulin. The use of cadaveric islets for transplants is limited to its quality during islet isolation and purification. Furthermore, donor islets are in short supply relative to the demand necessary for patients that would benefit from an islet transplant.

Implantation of stem cell derived pancreatic progenitors, stage 4 cells (S4), has been proposed as a treatment for diabetes. Implanted S4 cells require several months of maturation in the patient before they become functional. We use a step-wise 7 stage protocol to coax human embryonic stem cells into becoming stage 7 (S7) beta cells capable of producing insulin. S4 cells are first generated on a 2D culture system. The S4 cells express key transcription markers of pancreatic progenitors including PDX1 and NKX6.1. The S4 cells are then aggregated into islet-like clusters and further differentiated in a 3D culture system. In comparison to S4 implants, S7 implants require less time to become functionally mature, reversing diabetes in mice in weeks versus months. There is also less risk of the cells deviating from the desired fate (beta cells) since they are already matured at the time of implantation. Until now, no other studies have shown a characteristic dynamic response of stem cell derived beta cells to nutrients. By perifusion analysis, our cultured cells respond to a combination of glucose and the gut incretin mimetic Exendin-4.

Our modified S7 differentiation protocol shows promising results towards making mature beta cells. With further optimization, we hope to generate fully mature human beta cells that have insulin secretion kinetics similar to human islets. These cells could be a promising alternative to islets for diabetes treatment.

#8

Silencing of podocalyxin in developing mouse podocytes causes focal segmental glomerulosclerosis and proteinuria

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The rational design of podocyte-sparing therapies is hindered by our current limited understanding of podocyte biology. We therefore conducted a genetic loss of function study on the podocalyxin gene in developing mouse podocytes to better define its role in ultrafiltration. Using Cre-mediated recombination, we deleted *Podxl* from capillary loop stage podocytes *in vivo.* We expected this strain to recapitulate the perinatal lethality caused by anuria and hypertension previously observed in $Podxl^{-/-}$ mice, though our data curiously show a discordant phenotype. Newborn $Podxl^{\Delta POD}$ mice are capable of kidney filtration, producing urine at birth. Furthermore, podocytes in this strain develop normally, but their structure and function progressively deteriorates in the first weeks of life, resulting in focal segmental glomerulosclerosis (FSGS). In the absence of podocalyxin, foot process and slit diaphragm proteins mislocalize to the apical domains of podocyte cell bodies. Indeed, this correlates with a loss of ~4.4 p57+ glomerular cells in $Podxl^{\Delta POD}$ mice, indicating a loss of terminally differentiated podocytes from the glomerular capillary tuft. Our data suggests that the developmental time point during which podocalyxin mutations become expressed is a variable that determines whether anuria of proteinuria will manifest following the completion of nephrogenesis.

#9

Assessment of Neuro-2a cells as a model for characterizing reporters of neuronal catecholamine and ATP co-release

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Sympathetic innervation of blood vessels is a key regulator of vascular tone, which primarily contributes on physiological regulation of blood pressure. Sympathetic perivascular nerves simultaneously release several types of neurotransmitters, including adenosine-5'-triphosphate (ATP) and norepinephrine (NE). This phenomenon is referred to as co-release. There is an ongoing debate of how sympathetic neurotransmitters are spatially organized and released within the level of the single sympathetic nerve ending known as varicosity. Using immunohistochemistry, our lab has shown differential localization of clusters of vesicles containing ATP and NE in intact blood vessels and cultured superior cervical ganglia neurons. To further understand the physiological regulation of co-release, we developed genetically encoded reporters of the trafficking and release of vesicles containing the ATP and NE transporters, VNUT (SLC17A) and VMAT2 (SLC18A2). For the initial characterization of these probes, we sought a cell culture line that natively produced and released VMAT2 and VNUT containing vesicles. In this study, we examine the Neuro-2a (N2a), a dopaminergic mouse neuroblastoma cell line as a potential model of catecholamine and ATP co-release as a surrogate for primary sympathetic neurons. We assessed the degree of colocalization between pools of vesicles containing ATP and NE using genetically encoded fluorescent probes. Using NEBuilder cloning methods, we have created pH-sensitive reporters to characterize catecholaminergic and ATP vesicle trafficking and release, VMAT2-pHuji (a red pH-sensor) and VNUT-pHluorin. By elucidating the suitability of the cell model to meet our needs of reporter characterization, we further understand the differential regulation of co-transmission and address the fundamental physiological importance of sympathetic nervous system on blood pressure regulation.

#10

Granzyme B disrupts cell-cell adhesion and epithelial barrier function

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The skin is comprised of multiple layers of keratinocytes which together form a barrier to the external environment, regulating temperature, water loss, and pathogen exposure. As such the skin barrier is vital for health as well as disease prevention. Disruption of the epithelial barrier can result in infection, allergen exposure, and inflammation, culminating into severe conditions. Many autoimmune conditions, such as pemphigus, involve a dysregulation and accumulation of immune cells, this results in a disruption in skin barrier causing a loss of function. Granzyme B (GzmB) is a serine protease that is expressed and secreted by a variety of immune and non-immune cells. It can accumulate in the extracellular milieu and retain its proteolytic functions resulting in chronic inflammation and impaired tissue repair due to extracellular matrix (ECM) remodeling. As such, I hypothesized that GzmB disrupts epithelial barrier function through the proteolytic cleavage of cell junction proteins. The present study investigated the impact of GzmB on epithelial barrier dysfunction using Electric Cell-substrate Impedance Sensing (ECIS) and western blot analyses of intercellular junction cleavage fragments. Human formalin fixed, paraffin embedded blistered skin tissue was assessed for the presence of GzmB. GzmB treatment resulted in a loss of E-cadherin staining on the cell membrane which was supported by western blot analysis of the cell supernatants. Additionally, we observed a dose-dependent increase in E-cadherin fragmentation in GzmB-treated cells compared to controls. HaCaT cells exhibited a significant decrease in barrier function when treated with GzmB while cells treated with GzmB in the presence of a specific GzmB inhibitor remained unaffected. While absent in normal skin, GzmB was observed in abundance within the intra-epidermal blister in addition to the surrounding epithelium. In summary, GzmB contributes to a decline in epithelial barrier function in part through the proteolytic cleavage of cell-cell junctions.

#11

Characterization of hematopoietic cells generated from normal and leukemic human induced pluripotent stem cells (hiPSCs) in teratomas

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The elucidation of mechanisms that determine the properties of normal and leukemic human hematopoietic stem cells (hHSCs) has been revolutionized by advances in cell separation, clonal tracking, and the generation of new strains of highly immunodeficient mice. However, methods able to generate significant numbers of transplantable hHSCs *in vitro* either directly from patients or from hiPSCs have not yet been devised. Here we report the types of cells we have found can now be routinely formed in teratomas generated from $5x10^5$ hiPSCs transplanted subcutaneously ± mouse fibroblasts engineered to produce hFLT3-L, hSCF, hIL3 into immunodeficient NOD-*Rag1*-null- *IL2Rgc*-null mice with a c-kit deficiency (NRG-W41 mice) with or without an endogenous source of transgenically produced hIL3, hGM-CSF and hSCF (NRG-W41-3GS mice). Six to eight weeks post-transplant, the teratomas generated were dissociated into single cell suspensions and analyzed by FACS with various human-specific antibodies. Initial experiments demonstrated the presence of CD34+ cells in all teratomas with >0.1% CD45+ cells. Teratomas generated in NRG-W41-3GS mice from different iPSC lines produced ~40-fold more CD34+CD45+ cells than those generated in NRG-W41 mice, with yields of up to $7x10^6$ CD45+ cells (2% of total live cells in the teratoma). Co-injection of human growth factor (GF) producing mouse fibroblasts further increased the overall production of human CD45+CD34+ cells ~6-fold in the NRG-W41-3GS mice. Within this subset, we also identified GPI80+ and CD49f+CD38-CD45RA-CD90+ cells. Teratomas generated from both normal and CML derived hiPSCs were able to produce granulopoietic, erythroid and mixed colonies in standard GF-supplemented methylcellulose cultures providing evidence of functionally competent human hematopoietic progenitors. These experiments lay the foundation for a more detailed investigation of the conditions required for the generation of normal and diseased HSCs and their properties.

#12

Attenuation of the immune cell-secreted serine protease granzyme B reduces tissue damage and improves functional recovery following spinal cord injury

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Introduction: Spinal cord injuries (SCI) are traumatic events that can result in temporary or permanent loss of sensory and motor functions below the level of injury. Neural and glial cell death occurs during SCI in two main phases: the initial mechanical trauma (termed primary injury) and the resulting inflammatory, glial and vascular changes that exacerbate the initial tissue damage (secondary injury). Granzyme B (GzmB) is a serine protease well known for its role in cytotoxic T-lymphocyte mediated apoptosis. It is now recognized that GzmB can contribute to tissue destruction through extracellular protease activity. In the present study we characterized the effects of GzmB knockout on the functional recovery of mice subjected to SCI.

Materials and Methods: 3-Month-old GzmB Knockout (KO) or C57Bl/6 (wild-type) mice were subjected to a thoracic spinal cord injury and monitored for 1 or 6 weeks post injury. Locomotive function was assessed in injured mice using the Basso Mouse Scale, Horizontal ladder and Rotarod tests. Spinal cords were collected for histology and protein/RNA isolation. Eriochrome Cyanine R (EC) was used to quantify the amount of white matter in the injured spinal cords. Antibodies against Fibronectin, an extracellular matric (ECM) protein associated with fibrotic scarring, were used to assess extracellular GzmB activity in SCI lesions. qPCR was run on 1-week post injury mice to look at the expression of TNF-α and IL-1β.

Results: There is an increase in cellular GzmB expression in spinal cord lesions of mice at 5 days post injury. In addition, the global knockout of GzmB improved mouse locomotive scores following SCI. This was associated with an increase in white matter sparing at the epicenter and rostral to the injury. However, this reduction in tissue damage was not accompanied by a change in pro-inflammatory cytokine or ECM expression.

Conclusion: GzmB may mediate tissue damage and loss of function in spinal cord injury as an inflammatory effector of neuron death and demyelination. As such, GzmB is a novel therapeutic target that warrants further investigation for the treatment of spinal cord-related injuries.

#13

Biological characterization of distinct cell populations through NGS transcriptomics and epigenetics

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BRC-Seq is a Next Generation Sequencing Core service provider for RNA and DNA Illumina sequencing projects. Located at the Biomedical Research Centre at the University of British Columbia, we strive to make the power of sequencing available to all researchers at UBC and beyond. We offer services utilizing our Illumina NextSeq & MiSeq, 10x Genomics Single Cell Controller & eppendorf epMotion, as well as the knowledge and experience to deliver quality data.

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#14

A new model of de novo AML from c-MYC-transduced normal human CD34+ hematopoietic cells

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c-MYC is well known for its multiplicity of roles in normal cells and its increased expression in many human hematological malignancies, including some human acute myeloid leukemias (AMLs). But how and when deregulated c-MYC contributes to their genesis is not known. We have found that *c-MYC*-transduced CD34+ cord blood or adult bone marrow cells consistently produce a rapidly fatal (within 6-10 weeks) and serially transplantable human AML in immunodeficient mice transgenically engineered to produce 3 human-specific growth factors (IL-3, GM-CSF and Steel factor, 3GS). Interestingly, experiments using the parental strain of immunodeficient mice (i.e., not producing human 3GS) as hosts showed engraftment of the transduced cells, but no evidence of leukemogenesis for >8 months of follow-up. In 3GS mice, a phenotypically indistinguishable AML is produced from multiple subsets of *c-MYC*-transduced CD34+ cord blood cells including late granulopoietic-restricted progenitors. *In vitro*, *c-MYC*-transduced CD34+ cord blood cells were also factor-dependent but produced 7-fold larger clones than controls after 12 days, and rapidly outcompeted control cells in long-term cultures containing mouse stromal cells producing human 3S and other growth factors. This model offers a new system to elucidate and target early events that contribute to the development of human AML.

#15

Effects of the familial hypertrophic cardiomyopathy-related cardiac troponin T mutation I79N in biochemical systems of increasing complexity

Alison Yueh Li*, Sanam Shafaattalab*, Eric Lin, Bo Liang, Marvin Gunawan, Kaveh Rayani, Tiffany Barszczewski, Jonathan Davis and Glen F Tibbits

*These authors contributed equally to this abstract

Cardiac troponin (cTn) is a heterotrimeric complex that plays an essential role in cardiac contractility. Each complex is composed of a highly conserved Ca^{2+} binding subunit (cTnC), an inhibitory subunit (cTnI), and a tropomyosin binding subunit (cTnT). Familial hypertrophic cardiomyopathy (FHC) is the most common inherited cardiomyopathy, and 7% of FHC is associated with mutations found in the cTnT gene. Unlike the general anatomical abnormalities found in FHC patients, hearts from patients harbouring cTnT mutations show less ventricular hypertrophy but significant arrhythmogenesis. This study focuses on the I79N cTnT mutation that is morphologically asymptomatic but associated with a high incidence of sudden cardiac death.

The biophysical properties of the I79N mutation were investigated in reconstituted thin filaments (RTF) comprised of human recombinant proteins and skinned cardiomyocytes containing this mutation. At the RTF level, the mutation significantly slows the Ca^{2+} dissociation rate (80 s^{-1}) compared to the WT (102 s^{-1}) ($p<0.05$). In addition, higher myofilament Ca^{2+} sensitivity was observed for the cardiomyocytes reconstituted with human I79N cTn as demonstrated through a leftward shift of the pCa curve with ΔpCa of 0.65 ($p<0.05$). This is in agreement with the literature, in which the I79N cTnT mutation increases myofilament Ca^{2+} sensitivity and causes higher susceptibility to cardiac arrhythmia in transgenic mice.

The I79N TnT mutation was also incorporated into hiPSC-CM by genome editing, and the cells were characterized by optical mapping. Simultaneous voltage and Ca^{2+} recordings, using potentiometric (RH-237) and Ca^{2+} indicator (Rhod-2) dyes, were used to quantify the rates of spontaneous activity, action-potential profiles, and Ca^{2+} transient dynamics. The mutant hiPSC-CMs exhibited AP remodelling and triangulation which is considered a predictor of arrhythmogenicity. However, we did not observe significant Ca^{2+} transient prolongation in I79N *TNNT*$^{+/-}$ compared to WT hiPSC-CMs.

#16

Activation of fibro-adipogenic progenitors is a key pathogenic event in arrhythmogenic cardiomyopathy

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Arrhythmogenic cardiomyopathy (AC) is an inherited cardiac disease associated with fatal ventricular arrhythmias and sudden death, especially in young athletes. The pathophysiology of the disease is not clear, but it is believed that cardiomyocyte death, due to mutations in desmosomal proteins, activates a poorly defined population of progenitor cells resulting in their differentiation into fibrous/fibrofatty infiltrates causing life-threatening arrhythmias. Our lab and others identified a population of multipotent mesenchymal progenitors (fibro-adipogenic progenitors; FAPs) resident in the heart that express platelet-derived growth factor receptor alpha (PDGFRα) and stem cell marker Sca-1. These cells respond to damage by marked proliferation and differentiation at the injury site.

Using lineage tracing with PDGFRaCre^{ERT2}/tomato, we found that FAP fate was injury model-dependent. In ischemic injury, FAPs differentiated only along the fibrogenic lineage to a phenotypically distinct fibroblast population responsible for the formation of fibrous tissue replacing damaged myocardium. However, in AC, they generated intramyocardial mature adipocyte clusters as well, leading to the pathognomonic fibrous/fatty lesions. Interestingly, FAP activation in the absence of damage, by deletion of a cell cycle repressing transcription factor specific to mesenchymal progenitors, Hic1, was *per se* sufficient to produce a cardiac phenotype that mimicked AC in humans. Additionally, breeding a murine AC model with Hic1 KO mice resulted in a markedly accelerated AC phenotype. Finally, pharmacologic inhibition of FAP differentiation significantly improved cardiac function and mitigated post-injury remodeling. This suggests that, *independent of cardiac damage*, activation of FAPs is a key pathogenic event that could be targeted for treatment of AC.

#17

The nature of the hot spot for tissue destruction in COPD

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RATIONALE

Previous reports from our laboratory have shown small airways disease is associated with an innate and adaptive inflammatory immune response and that it is associated with the massive destruction of terminal bronchioles before emphysema can be recognized on routine thoracic CT scans. The present report identifies a 'hot spot" for terminal bronchiolar destruction in COPD and describes the gene expression profiles associated with it.

METHODS

Gene expression profiling, MicroCT, and quantitative histology were performed on 40 samples from each of 5 PLE, 5 CLE, and 5 control lungs respectively. The immune network was manually collected from distinct public databases on the basis of GO annotation. Cibersort & MMC are run on the expression profile to further validate our discoveries.

RESULTS

The data presented here identify a hot spot region for the destruction of terminal bronchioles that are located in regions of the lung where the mean linear intercept (Lm) used to estimate the severity of emphysematous destruction, ranges from within its normal range of 250- 500 µm. To the lower limit of the spatial resolution of modern clinical CT scanners which is approximately 1000 µm. Importantly these data also provide molecular evidence of the activation of adaptive immunity within this "Hot spot" that includes IFNG activation, T cell recruitment, as well as the expression pattern of immune checkpoint genes within the hot spot region. The long-term goal of this project is to discover the key molecular mechanisms involved in the destruction of terminal bronchioles and alveolar tissue in the hot spot region, which represents the earliest stages of COPD.

CONCLUSIONS

The present results both confirm and extend our previously histological data-based discovery that the adaptive immune response has been already activated in COPD. Validated by the latest histological observations, the current results on the basis of both gene expression profiling and Cibersort prediction demonstrates the activation of adaptive immunity in the hot spot region, where the destruction of terminal bronchioles is well advanced but emphysematous destruction cannot be identified on the MDCT scans. The activation of the adaptive immune system might initiate the terminal bronchioles destruction in COPD.

#18

Role of TGFβ-Activated Kinase 1 in muscle-resident fibro/adipogenic progenitors, a key modulator of the inflammatory environment

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Fibrosis is a particular concern in degenerative myopathies, as it is the clinical parameter that best correlates with loss of muscle strength. Others and we have identified changes in the inflammatory milieu taking place in muscular dystrophies as a key mechanism underlying fibrosis development. Inflammatory cell-derived factors modulate the survival of Fibro/Adipocyte Progenitors (FAPs) as well as their ability to acquire a fibrogenic phenotype, at the same time limiting the activity of the myogenic progenitors driving regeneration. Conversely, activated FAPs are the main source of cytokines and chemokines modulating the inflammatory environment following muscle damage. We identified TGFβ-Activated Kinase 1 (TAK1) as a key signal transducer involved in these processes. TAK1 was specifically deleted in adult FAP using a Cre-ERT2 system. Damage was performed by injection of Notexin in *Tibialis Anterior* (TA) muscles of control and TAK1-KO mice.

Compared to control, skeletal muscle regeneration is strongly delayed in the absence of TAK1 in FAPs. Indeed, mass of TA muscle, size of damaged myofibers and number of nuclei per fiber are reduced at 7 and 14 days after damage (d.p.n.). Associated to these defects, proliferation of both MPs and FAPs was significantly decreased. In this acute damage model, all parameters normalized by 28 d.p.d., suggesting that TAK1 modulates early steps of regeneration, which are known to be influenced by the inflammatory environment. Indeed, while there was no difference in macrophage infiltration and phenotype, we detected a massive increase in a specific subset of immune cells ($CD3/NK1.1^{-}Ly6C/G^{-}CD45^{+}F4/80^{-/low}CD11b^{low}$), which we characterized as eosinophils ($SiglecF^{+}$). Analysis of RNA sequencing data from activated TAK1-KO FAPs reveals the up-regulation of a specific chemokine implicated in eosinophil migration and infiltration.

Eosinophilia has been reported as a component of Duchenne muscular dystrophy, and these cells are particularly enriched in muscles that develop fibrosis. Thus this chemokine could be a potential therapeutic target for muscle dystrophies.

To conclude, our central hypothesis is that TAK1 in FAPs is a key-signaling node regulating cytokine secretion, and therefore governing the establishment of the inflammatory milieu in damaged muscle, which itself modulates MP fate and skeletal muscle regeneration.

#19

Topical small-molecule GzmB inhibitor treatment improves burn wound healing in diabetic mice

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Background: Worldwide, there are about 350 million diabetes patients and approximately 15% of these patients will develop diabetic foot ulcers during their lifetime. Diabetic wounds, especially diabetic foot ulcers, are one of the major complications of diabetes. Unfortunately, effective pharmacological interventions aimed at improving diabetic wound healing are not available and the current treatment for diabetic wounds mainly comprises conventional wound treatment with different types of dressings and bandages. Granzyme B (GzmB) is a member of granzymes, a serine protease family with a broad substrate spectrum. Previous studies have shown that GzmB degrades many extracellular matrix proteins that are vital for facilitating wound closure, reducing scar formation and restoring skin tensile strength. Inhibition of GzmB by SerpinA3N accelerates wound healing in diabetic mice. However, SerpinA3N is a murine protein with a broad spectrum of substrates, which makes it unsuitable for further clinical use. As such, it is essential to investigate the therapeutic efficacy of small-molecule GzmB inhibitor in a murine model of diabetic wound healing.

Methods: Degree 2a-2b burns (1 cm diameter) were induced on the dorsal side of diabetic mice (aged 10-12 weeks). After the injury, the animals were treated every day with topical application of GzmB inhibitor in gel form or with vehicle gel for 30 days. Standardized photos were taken every 3 days. Photographs were analyzed by tracing; percent wound closure was calculated against the size of the original wound area for each animal. Mice were sacrificed at 30 days after injury and skin samples were collected for histology and biophysics analysis.

Results: Topical GzmB inhibition significantly attenuated the initial burn wound expansion and accelerated wound closure in diabetic mice. Further histology showed a thinner epidermal layer, stronger decorin staining and greater collagen density and organization in GzmB inhibitor-treated groups compared to the vehicle control group. GzmB inhibitor-treated wounds also exhibited greater tensile strength compared to controls, suggesting that GzmB inhibition improves tissue remodeling.

Conclusion: Topical GzmB inhibition improves wound closure and tissue remodeling in diabetic burn wounds.

#20

Transcript and protein expression profile of hiPSC derived atrial like cardiomyocytes

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The advent of pluripotent stem cell (PSC) derived cardiomyocytes has revolutionized the field of cardiac research. For the first time, the study of human disease in human models is possible without the difficulty of obtaining biopsy tissue. Additionally, the use of patient derived stem cells (iPSCs) has allowed for the study of a patient's disease in a personalized manner. Current differentiation protocols result in a mixed cardiac population that consists of mostly ventricular cells. This makes the study of region specific diseases difficult. In particular, the world's most common arrhythmia, atrial fibrillation (AF) cannot be accurately studied without atrial cells. As such, the development of subtype specific differentiation protocols is vital. Retinoic acid has long been identified as a modulator of cardiac subtypes in animal models. The enhancement or inhibition of retinoid pathways has resulted in the respective enlargement or shrinkage of the atria in chick embryos. Recent work has shown that the addition of retinoic acid during the cardiac mesoderm phase in embryonic stem cells increased atrial lineage significantly. These differentiations however, were completed using ActivinA/BMP (Act/BMP) protocols. Act/BMP protocols require precise titrations for individual cell lines and as a result can be difficult to use. They are also costly to run. Here, the lessons learned from previous protocols were applied to a more accessible CHIR (GiWi) protocol. We hypothesized that the addition of retinoic acid during the days in which cells are in the cardiac committed mesoderm phase, days 3-5, will steer them towards an atrial like fate. At day 20, cells were harvested and analyzed by three different assays. The first, a quantitative real time PCR (qRT-PCR) assay, was used to discern differences in expression in chamber specific transcripts. The Second, a flow cytometry assay, determined the protein level expression changes in the cardiac marker troponin T (cTnT) and the ventricular marker, myosin light chain 2V (MLC2V).

#21

CRISPR/Cas9-mediated gene editing in *GBA1* and *NAGLU*: Investigating a regenerative therapy for Gaucher disease and Mucopolysaccharidosis IIIB

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Lysosomal storage diseases (LSDs) are rare genetic disorders caused by a dysregulation of lysosomal function. Gaucher disease (GD) and mucopolysaccharidosis IIIB (MPS IIIB) are two LSDs resulting from reduced function of glucocerebrosidase (GCase) and α-N-acetylglucosaminidase (Naglu), respectively. GD results from mutations in *GBA1,* and MPS IIIB from mutations in *NAGLU.* Both diseases are characterized by neurodegeneration, resulting in premature lethality. Moreover, substantial evidence indicates that mutations in *GBA1* increase an individual's risk 4-6 fold for developing the second most common neurodegenerative disease, Parkinson's disease, later in life. Common treatments for LSDs include recombinant enzyme replacement therapy. However, recombinant GCase and Naglu fail to cross the blood-brain barrier and resolve progressive neurodegeneration in GD and MPS IIIB patients.

Gene editing of *GBA1* and *NAGLU* in induced pluripotent stem cells (iPSCs) offers a potential solution to the lack of treatment options in GD and MPS IIIB. We have generated iPSCs from GD and MPS IIIB human skin fibroblasts using Sendai viral vectors that transiently express transcription factors c-Myc, Klf4, Oct3/4, and Sox2. We have utilized the clustered regularly interspaced short palindromic repeats (CRISPR)/ CRISPR-associated endonuclease 9 (Cas9) to target and edit *NAGLU* and *GBA1* genes. We performed *in vitro* RNA-guided engineered nuclease (RGEN)-restriction fragment length polymorphisms (RFLPs) using 15 guide RNAs (gRNAs), followed by detection of gRNA targeting efficiency in iPSCs and fibroblasts. Correction templates were delivered with Cas9-gRNAs to drive homology directed repair. Edited iPS clones were screened using fluorescence microscopy, monoclonal isolation, and RFLP for gene-modified alleles. We will perform additional assays to identify corrected iPS clones using the highly sensitive droplet digital PCR assays. In summary, we have targeted *GBA1* and *NAGLU* mutations for gene editing in iPSCs as a proof-of-concept method to restore normal enzyme function in patient-derived cells. This method of gene editing in iPSCs is a putative regenerative therapy for patients afflicted with LSDs.

#22

Culture and differentiation of mouse hepatic organoids using Hepaticult™ serum-free media

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Liver organoids are miniature three-dimensional (3D) cell culture systems for studying liver cell biology. Liver organoids retain many features of *in vivo* hepatocytes, including a polarized epithelium, and represents a more physiological system than conventional 2-dimensional cell culture for studying hepatic development, regeneration, detoxification, metabolism, and disease. We have developed a novel, serum-free HepatiCult™ Organoid Growth Medium and protocols for establishing and expanding hepatic progenitor organoids derived from mouse liver tissue. Mouse livers were enzymatically treated to isolate the putative liver stem cell niche contained in hepatic ducts. The ducts were then further dissociated into single cells to derive clonal organoids. Liver organoids formed within 4 - 7 days from hepatic ducts or single cells that were embedded in Corning® Matrigel® and cultured in HepatiCult™ (*n* = 148 mice). The organoids were passaged every 5 - 7 days at split ratios between 1:10 and 1:30 and could be maintained in culture for > 2 years, indicating the presence of self-renewing hepatic stem cells. Cells within the organoids expressed genetic markers representative of hepatic stem and progenitor cells (*Prom1*, *Axin2*, *Sox9*, *Cd44*), ductal cells (*Krt19*, *Hnf1b*) and hepatocytes (*Hnf4a*, *Afp*), and were primed for downstream differentiation into mature functional hepatocytes. Differentiation was easily induced using a published protocol (Huch *et al*., *Nature* 2013). Production of the organoids could be scaled up by culturing them on an orbital shaker in a dilute suspension of Matrigel® in HepatiCult™. Methods were also established to cryopreserve the organoids for long-term storage. Our results demonstrate that HepatiCult™ promotes the establishment, expansion, long-term propagation and banking of mouse hepatic organoids that maintain their capacity for differentiation.

#23

A feeder-independent culture system to convert and maintain human pluripotent stem cells in a naïve-like state

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Human pluripotent stem cells (hPSCs) within the developing embryo progress through a spectrum of pluripotent states as they transition from naïve to a more lineage-restricted, primed state. Capturing these states *in vitro* requires specialized culture media and protocols. To date, conditions that maintain hPSCs in naïve-like states have depended on the use of feeder cells for robust long-term expansion. RSeT™ Feeder-Free (RSeT™-FF) is a defined medium that supports the reversion of primed hPSCs to a naïve-like state and supports their long-term feeder-independent maintenance. To revert primed hPSCs cultured in mTeSR™1 or TeSR™-E8™, colonies were seeded as small clumps on Corning Matrigel® coated plates. After 24 hours, the medium was changed to RSeT™-FF supplemented with 0.2% Matrigel® and hPSCs were cultured for 5 days with medium changes every other day. hPSCs maintained in RSeT™-FF + 0.2% Matrigel were dissociated to single cells and re-seeded at a density of 2.1×10^4 cells/cm^2 on Matrigel®-coated plates every 4-6 days. Colony morphology and cell expansion was assessed at each passage (P). Transition to a naïve-like colony morphology was observed in cultures as early as P1, with colonies possessing a highly homogeneous naïve-like morphology with >90% of colonies displaying a domed morphology, and extremely low levels of background differentiation (n=5 hPSC lines). On average, hPSCs cultured in RSeT™-FF expand by 3.0 ± 0.9-fold per passage (n=4); comparable to hPSCs cultured in RSeT™ Medium on feeders. Cells from naïve-like colonies (>P5) expressed markers associated with undifferentiated hPSC such as OCT4, SSEA4, TRA-1-60 and ALP. Importantly, as in RSeT™ Medium on feeders, naïve-like hPSCs cultured in RSeT™-FF Medium showed upregulation of genes commonly enriched in the naïve-state (*KLF2*, *KLF4*, *KLF17*, *TFCP2L1*, *STELLA*, and DNMT3L1) compared to mTeSR™1-cultured primed hPSCs. Additionally, naïve-like hPSCs maintained in RSeT™-FF Medium are capable of direct differentiation to all somatic lineages using the STEMdiff™ Definitive Endoderm, Mesoderm Induction, and SMADi Neural Induction Kits. In summary, we have developed RSeT™-FF, a defined medium that promotes robust conversion of primed hPSCs to a naïve-like state and the continuous maintenance of these cells in a feeder-independent culture system.

#24

Patient specific iPSC derived cardiomyocytes as a model for doxorubicin induced cardiotoxicity

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Introduction: Doxorubicn is a chemotherapy drug, used to treat a variety of malignancies including breast cancer, sarcoma and lymphoma. However, some patients experience cardiotoxicity that can lead to heart failure and death. Genome wide association studies have identified a genetic variant in the *RARG* gene that is associated with increased susceptible to doxorubicin induced cardiotoxicity (DIC). However, the functional role of *RARG* in DIC is unknown. The goal of this study was to investigate the role of *RARG* in DIC using induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs).

Methods: We generated iPSCs from doxorubicin treated patients who experienced DIC and control patients who did not experience toxicity. We used CRISPR genome editing to edit the RARG gene. We differentiated the iPSCs to generate CMs and treated them with doxorubicin. We assessed doxorubicin toxicity with various cellular and electrophysiological assays.

Results: Doxorubicin treatment caused dose-dependent cell death in iPSC-CMs. iPSC-CMs from DIC cases were significantly more sensitive to the toxic of doxorubicin in vitro compared to iPSC-CMs from controls (LD50: 0.7658µM and 3.689µM respectively, $p<0.0001$). Using optical mapping, we showed that doxorubicin caused action potential duration shortening, an effect that was more pronounced in iPSC-CMs from cases. We also used CRISPR to inactivate RARG. RARG-Knock Out (KO) iPSC-CMs were less susceptible to DIC, showing increased cell viability with doxorubicin, compared to isogenic control cells. This finding was replicated in embryonic stem cell (ESC)- derived CM where we deleted the RARG-KO.

Conclusion: iPSC-CMs from patients that experienced DIC are significantly more susceptible to doxorubicin than iPSC-CMs from control patients. Our results show protection from DIC in cells with deletion of the *RARG* gene, supporting a causal role of this gene in DIC.

#25

Using CFD to maintain stem cell aggregate size during bioreactor scale-up

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Stirred suspension bioreactors offer several advantages over planar static culture, making them an increasingly popular platform for stem cell expansion and scale-up. A change in bioreactor scale, however, results in altered hydrodynamic conditions which affect cell products - particularly if grown as aggregates. Shear stress from the local fluid velocity gradient and turbulent eddies influence aggregate sizes, which in turn impact cell pluripotency, differentiation, and proliferation potential. Current scale-up methods rely on measures which do not capture the variation and complexity of the flow field generated during stirred suspension bioreactor operation. Depending on the scale-up method used, predicted impeller agitation rates can differ greatly, resulting in significant differences in cell growth. This study evaluates the use of a computational fluid dynamic (CFD) based scale-up method to predict agitation rates that maintain aggregate size between bioreactors of various sizes and geometries. ANSYS Fluent CFD software was used to simulate fluid flow in 10-mL, 100-mL, and 500-mL computer controlled stirred suspension bioreactors. The CFD model was used to calculate the volume average turbulent dissipation rate and volume average shear stress for each bioreactor across a range of agitation rates. The obtained volume average turbulent dissipation rate and shear stress were used to predict agitation rates during scale-up. Mouse embryonic stem cells (mESCs) were cultured using these predicted agitation rates and resulting aggregates were sized using a custom ImageJ software plugin to analyze microscope images. It was determined that maintaining constant volume average turbulent energy dissipation rate throughout scale-up preserved aggregate size. This held true when scaling to stirred suspension bioreactors of different volumes and geometries. Accurately predicting agitation rates with CFD to maintain aggregate sizes enables efficient and cost-effective scale-up of stem cells without extensive experimental testing of bioreactor agitation rates.

#26

An integrated high-throughput microfluidic platform to fabricate well distributed cell-laden gelatin methacryloyl microgels

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Introduction

Tissue engineering applies a combination of material, mechanical, chemical and biological sciences with the aim of creating systems that are able to repair of even replace a biological function. Microfluidics principles have been utilized as powerful tools to fabricate controlled monodisperse cell-laden hydrogel droplets for various biomedical applications, most importantly for tissue engineering applications.

Materials and Methods

A flow focusing microfluidic device was fabricated by applying photolithography combined with softlithography methods. Gelatin methacryloyl (GelMA) prepolymer was synthesized from porcine skin gelatin and used to encapsulate cells. NIH 3T3 mouse fibroblast cells were mixed with GelMA prepolymer solution and the VA-086 photoinitiator.

Cells were treated with bovine serum albumin (BSA) before mixing with the GelMA prepolymer solution to prevent cell aggregation. A magnetic mixer was applied to a syringe to evenly distribute cells in the GelMA prepolymer solution. The prepolymer-cell mixture was then applied as the disperse phase to the flow focusing microfluidic device. A 405 nm laser was used as a light source illuminating over the crosslinking chamber of the device to crosslink the GelMA prepolymer. Finally, the microgels passed through the filter unit of the device where they were washed out from the oil and into a washing aqueous phase of PBS containing the biocompatible surfactant tween 80.

Results and Discussion

Encapsulating cells to hydrogel prepolymer droplets have been problematic for the stable droplet generation process with uniform microgel size. The problem is mainly due to the non-uniform distribution of cells throughout the hydrogel solution because of the cellular sedimentation and aggregation. As well, off-chip performance of both the crosslinking and oil separation processes reduces the throughput and affects cellular viability.

Applying the magnetic mixer and BSA could reduce cellular aggregation during the droplet generation process. And hence, reasonable number of cells were encapsulated in GelMA droplets. The BSA occupied cellular receptors responsible for prohibiting cell aggregation. While the magnetic micromixer evaded cells from settling down by the effect of gravity during the microdroplets formation process. A 405 nm laser was applied for fast on chip photocrosslinking of cell-encapsulated GelMA droplets (less than 30 seconds). In the filter unit, tween 80

surfactant simplified the extraction of the microgels out from oil. These combined approaches resulted in a cellular viability of around 85% at day 1 and was maintained throughout 5 days.

Conclusion

Several microfluidic devices and techniques were previously used to produce cell-laden hydrogel droplets. However, a uniform distribution of cells among the generated droplets was difficult to achieve. In addition, gelation and oil separation steps are usually harsh for the cells using ultraviolet light and strong centrifugation, respectively. Also, these steps require a relatively long time and were undergone off-chip in separate steps, which makes the throughput very low for any subsequent practical application. A cell-laden hydrogel droplet generation in a continuous high-throughput way was established with a practical cellular distribution among uniform sized microgel droplets. Incorporating the on chip fast crosslinking and filtration processes enabled a high throughput generation of cell-laden droplets with high cellular viability.

#27

Visible light based stereolithography 3D bioprinting system for long duration biofabrication

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In the present decade, tissue engineering research has become highly focused on 3D bioprinting of tissues. This focus is driven by the requirement of rapid and efficient fabrication of cell-laden scaffolds for application as high throughput assays and in-vivo models. The scaffolds fabricated with various 3D bioprinting methods can mimic the *in vivo* microenvironment with varying degrees. Stereolithography has emerged as one of the significant bioprinting methods for rapid fabrication of complex 3D scaffolds supporting cell encapsulation. Most of the present systems utilize UV light (UV-A, 365nm) for crosslinking the methacrylated hydrogel in presence of a phototoinitiator. Some systems also utilize near UV light (405nm) for crosslinking and fabricating the scaffold. Although, stereolithography bioprinting is faster than other common bioprinting methods, fabrication of human scale tissues can take long hours. Long duration exposure to UV and near-UV lights have been found to be harmful for cells and hence, there is a need to restrict the use of UV light for bioprinting. To address this need, we present a visible light based stereolithography bioprinting system using Gelatin methacryloyl (GelMA) and Eosin Y (EY-GelMA) operating within the visible light spectrum. Through systematic experiments, we developed the bioprinting system and compared it with the commonly used UV light based stereolithography system using Irgacure and GelMA. The cell viability is similar for day 1 and day 6 for both systems. EY-GelMA exhibited more cell attachment and elongation on day 6. Further, we characterized our system by comparing mechanical properties and cell viability with the Irgacure-GelMA system (Irgacure 2959 as photoinitiator). In order to optimize the bioprinting process of the developed system, the effect of GelMA and Eosin-Y concentration on the mechanical strength was examined and biocompatibility of the crosslinked EY-GelMA. Next, we will investigate the time required for crosslinking of EY-GelMA for various concentrations of Eosin-Y and GelMA in order to optimize the bioprinting process. Our observations for studying the effect of generated free radicals and the exposure light on the cell viability demonstrate significant decrease in cell viability for Irgacure-GelMA system in the presence of free radicals and UV-A exposure for a long duration (~1 hour). On the other hand, the EY-GelMA based visible light bioprinting system exhibited higher cell viability for long term exposure without any significant difference which points towards more feasibility and compatibility of the presented visible light stereolithography system for long duration bioprinting processes.

#28

A stretchable 3D cellular microarray for mechanobiology

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Cells *in vivo* are subjected to mechanical signals including deformations, external mechanical forces, and the stiffness of the extracellular matrix[1]. These stimuli play a crucial role in regulating cellular phenotype and genotype, and numerous reports studying the effect of strain field application and matrix stiffness on cellular behavior have been well established[2]. However, most of these studies are based on two-dimensional (2D) *in vitro* models which fail to mimic the microenvironments found in the body. Cell behavior in three dimensions (3D) differs completely from that in 2D[3]. It is therefore vital to study the cell responses to mechanical stimuli in a 3D environment. 3D miniaturized cell culture platforms have been generated using microarray bioprinting technology, however few efforts have been made to study the effects of mechanical cues on cellular microarrays. Recently developed platforms for studying the effect of mechanical cues in 3D neither offer high throughput nor evaluate multiple mechanical cues. To address this need, we report a deformable, high throughput platform that allows 3D dynamic stretching of cell-laden biomaterial microarray constructs.

Our approach involves the use of an inkjet 3D bioprinter to form an array of photocrosslinked microgels on an elastomeric polydimethylsiloxane (PDMS) membrane. An intermediate layer of polyacrylamide gel is grafted to the PDMS membrane through benzophenone to permanently bond the crosslinked microgels to PDMS membrane and facilitate complete strain transfer from PDMS to microgels. A 15x5 microarray of Gelatin Methacryloyl (GelMA) prepolymer solution encapsulated with cells was printed on to the developed substrate. Printed cells within the microgel construct had high cell viability right after printing and even after 7 days of culturing. Attachment, elongation and proliferation of cells within GelMA microgel was also clearly seen through the 3D networks formed by them after 5 days of culturing. Similar substrate printed with an array of cell-laden microgels was subjected to 10% strain at 1 HZ for 3 days. Cells, initially oriented in random, were seen to align along the stretching direction with enhanced proliferation within the GelMA microconstructs. Apart from studying cellular orientation, our system could be effectively used to select the biomaterials that promote the differentiation of stem cells when 3D stretched. Our platform can be scaled up to introduce a wide range of extracellular cues and screen the cell responses and tissue formation in vitro. We believe our microarray platform could be a potential device to screen different biomaterials that support cell growth and guide their function in the presence of mechanical cues for tissue engineering and regenerative medicine purposes.

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#29

Engineering neural tissue from human pluripotent stem cells using small molecule releasing microspheres

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Continuous exposure to specific morphogens can direct stem cells to differentiate into the desired cell types. Drug delivery systems made out of biodegradable and biocompatible polymers provide such exposure of the necessary drugs and promote homogeneous differentiation of stem cell aggregates. This study focuses on differentiating human induced pluripotent stem cells (hiPSCs) into cholinergic neurons using drug releasing microspheres. The resulting engineered tissues can be used as drug screening tools or as a potential treatment for spinal cord injury (SCI).

hiPSC aggregates were combined with purmorphamine (puro) and retinoic acid (RA) releasing microspheres to induce differentiation *in vitro* for 64 days. Cell aggregates were formed using AggreWell™ 800 inserts. After 7 days, aggregates were transferred into laminin-coated plates for further cell differentiation. Immunocytochemistry (ICC) and flow cytometry were performed to identify pluripotency markers (Sox2, SSEA-4), early neuronal markers such as Nestin (neural progenitor cells) and β-tubullin III (immature neurons), HB9 and ISL-1 (motor neurons), and mature markers such as ChaT (cholinergic neurons) O4 (oligodendrocytes), and Sy38 (synaptophysin). Neural length, branching, and physiological relevance of the tissues were also analyzed.

Cell aggregates combined with drug releasing microspheres showed neural rosette formation and expression of β-TIII by day 15 indicating neural differentiation. By day 35, cell aggregates showed significant neurite extension, organization as well as the expression of the motor neuron markers HB9 and IS-1. By day 60, ICC and flow cytometry analysis confirmed mature neural phenotypes as observed by the expression of ChaT. By day 64, spontaneous action potentials were observed confirming the maturation and physiological relevance of the differentiated tissues. In the future, hiPSC-derived neural progenitor cells will be bioprinted inside of a fibrin based-hydrogel along with drug releasing microspheres. Bioprinting of neural tissues will be performed using Aspect Biosystems' Lab-on-a-printer(LOP)™ technology.

#30

Analysis of lymphoid restriction in human hematopoietic cells defined by their clonal outputs in multi-lineage cultures

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Lifelong mature blood cell production can be sustained by rare human hematopoietic cells with extensive proliferative potential. However, the mechanisms that enable single lineages to be produced are still poorly understood. We are interested in defining how the potentialities for neutrophil/monocyte (NM) and lymphoid differentiation become segregated, given the physiological impact of this fate decision in normal cells and its inferred involvement in the genesis of different acute leukemias. To enable these two (and other) lineage potentialities to be detected in single cells with improved specificity and sensitivity, we developed a 3-week stromal + growth factor-based culture system optimized for the FACS detection of NM and B/NK-lymphoid cell outputs from single cord blood (CB) input cells covering the full range of conventionally defined CD34+ phenotypes. Assays of 360 single CD34+ CB cells in such cultures, showed 20% to 90% of the cells in any given subset produced a detectable clone (>50 cells after 3 weeks). As expected, we found some “late” phenotypes were enriched for progenitors of only lymphoid or only NM cells (73% in pre-B/NK cells and 76% in $CD38^{hi}$ fraction, respectively). However, a large proportion of progenitors in the CMP and GMP subsets, as well as in the “more primitive” CD34+CD38- population produced clones containing both NM and lymphoid cells. By comparing the molecular features of individual index-sorted members of each of these phenotypically defined compartments, we expect to identify shared features that correlate with their lineage outputs.

#31

Efficient enrichment of functional ILC subsets from human PBMC by immunomagnetic selection

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Innate lymphoid cells (ILCs) are exceedingly rare but important regulators of homeostatic and disease-associated immune processes. The frequency of ILCs in peripheral blood of healthy humans is ~0.07% of $CD45^+$ leukocytes. ILCs lack specific cell surface markers but can be divided into distinct subsets (ILC1, 2 and 3) based on their differential expression of effector cytokines and master transcription factors.

Currently, cell sorting is the most widely used method to isolate ILCs, but it is time consuming, expensive and often results in low purities and recoveries. Pre-enrichment of ILCs would allow for reduced sorting times and improved purities. Accordingly, we have developed a fast immunomagnetic negative selection method to pre-enrich all ILCs subsets from human leukapheresis samples. Briefly, unwanted cells are labelled with antibodies and magnetic particles and placed into an EasySep™ magnet. Unwanted cells are retained in the magnet and the enriched ILC fraction is simply poured off into a new tube. We find that total ILCs (defined as $Lineage^-$ $CD45^+$ $CD127^+$) are enriched from a frequency of 0.01 - 0.23% (n=28) to a final frequency of 17 - 86%, an enrichment of 200 - 1,500 fold with virtually no loss of ILCs. ILC1 were enriched from 0.01 - 0.2% to 4.5 - 14%. ILC2 were enriched from 0.01 - 0.1% to 5.8 - 51% and ILC3 were enriched from 0.01 - 0.1% to 6 - 16%. This pre-enrichment drastically decreases sort time, allowing for purification of 3.7×10^5 ILCs from 2×10^9 Peripheral Blood Mononuclear Cells (PBMCs) in only 12 minutes. Sorted cells maintained their functionality; when stimulated *in vitro* with cytokine cocktails, ILC1s produced IFNγ, ILC2s secreted IL-13 and ILC3s produced IL-22. Similarly, sorted ILCs in co-culture with intestinal organoids modulate gene expression of the intestinal cells. This newly developed method of ILC pre-enrichment will aid human ILC research by enabling their rapid isolation when combined with cell sorting.

#32

Expansion of mouse prostate epithelial stem cells in serum-free ProstaCult™ Organoid Growth Medium

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Growing prostate epithelial cells as organoids in a three-dimensional (3D) cell culture environment represents a more physiological model system than conventional 2D adherent cell culture systems for studying many different aspects of prostate epithelial cell biology (Karthaus et al, Cell 2014). We are currently developing ProstaCult™ Organoid Growth Medium, a serum-free medium for the long-term propagation of organoids from mouse prostate tissue. To initiate the cultures, mouse prostates are resected and enzymatically dissociated sequentially in collagenase type II, trypsin and dispase/DNase I to generate a single cell suspension, and 5 x 10^3 of the liberated cells are then embedded in Corning® Matrigel® and cultured in ProstaCult™ Organoid Growth Medium. Approximately 10 ± 2% (mean ± SEM; n = 6) of the seeded cells will proliferate and generate organoids that typically have a large cystic morphology. Immunostaining of these organoids reveals that they are composed of a polarized epithelium made up of androgen receptor (AR)- and keratin (K) 8-expressing luminal cells and K5-expressing basal cells (n = 12). Organoids can be passaged every 7 days, either as a single cell suspension or as small organoid fragments. Passaging cultures as single cells results in robust cell expansion (21-240-fold per passage), but cultures typically senesce between passages 5-7. Passaging organoids as small fragments at 1:5-1:20 split ratios demonstrates that cultures can be maintained for a minimum of 10 passages, while still maintaining multilineage potential. These results demonstrate that ProstaCult™ Organoid Growth Medium efficiently generates and expands prostate epithelial organoids.

#33

Heterozygous deletion of *KCNH2* models long QT type 2 in human pluripotent stem cell-derived cardiomyocytes

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Cardiac arrhythmias in the general population and within families are most frequently associated with heterozygous gene variants which include the long QT syndrome. Genome-editing of human induced pluripotent stem cells (hiPSC) can be used to model cardiac arrhythmias. *KCNH2* encodes for the hERG channel which is important for cardiomyocyte repolarization. It was previously reported that a heterozygous point mutation in the PAS domain of hERG was associated with familial long QT type 2 using an overexpression heterologous system. We hypothesized that single copy deletion of *KCNH2* by targeted gene-editing of the PAS domain would display the long QT phenotype in hiPSC-derived cardiomyocytes (hiPSC-CMs). We used CRISPR/Cas9 and a highly efficient cloning supplement (CloneR™) to edit *KCNH2* and derive a clonal hiPSC line. Control and edited isogenic hiPSC lines were differentiated to cardiomyocytes using an optimized cardiomyocyte differentiation media and protocol (STEMdiff™ Cardiomyocyte System). We generated a heterozygous four nucleotide deletion in exon 2 of *KCNH2* in a hiPSC line using CRISPR/Cas9. The deletion produced an early stop codon in the PAS domain located in the N-terminal region of the hERG channel. Single cell cloning efficiency was 20% for a seeding density of 7 hiPSCs per cm^2. The clonal control and edited isogenic hiPSC lines were differentiated to cardiomyocytes. The isogenic hiPSC-CMs expressed >80% cTnT. Immunocytochemistry and electrophysiological was performed on hiPSC-CMs 28 days after differentiation. hiPSC-CMs with the heterozygous hERG deletion had an expression pattern of the hERG channel protein that was restricted near the nucleus and these hiPSC-CMs showed prolonged field potential durations (FPDs) with an irregular excitability profile and beat period. The isogenic control hiPSC-CMs had shorter FPDs and a stable excitability profile. In conclusion, we determined that single copy deletion of *KCNH2* in hiPSC-CMs prolongs cardiomyocyte repolarization and generates arrhythmias characteristic of long QT syndrome.

#34

Pericytes: novel insights about their heterogeneity in adulthood from mouse to human

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The World Health Organization reports that cardiovascular diseases are the number one cause of death globally. Surgical revascularization is the most common intervention, however, not always successful or achievable. The mesenchymal stem cells (MSCs) therapeutic potential has been explored as an alternative or adjuvant angiogenic therapy, which aims at growing new blood vessels in the ischemic tissue. However, the results of individual trials involving MSCs are rarely in agreement. We believe part of this confusion stems from the underappreciated heterogeneity of mesenchymal progenitors. In fact, our results indicate that at least two distinct cardiac-resident perivascular mesenchymal progenitors reside in the heart: multipotent fibro/adipogenic progenitors, which generate new matrix and modulate inflammation; and pericyte likely involved in regenerative angiogenesis. Despite the richness of proposals regarding the embryonic origin of pericytes in different organs, much less is known about how pericytes are maintained and spread along growing vessels in adulthood. We tackled this performing Single Cell Sequencing on murine cardiac-pericytes purified based on a marker suggested by previous studies. The cluster analysis revealed two subgroups, one of which was associated with mature pericytic genes, and the second with progenitor-like genes. We took advantage of novel tracing systems we generated to address the relation between these pericyte subpopulations after myocardial infarction. We found that the putative progenitors increased the expression of mature pericytic markers and underwent proliferation more efficiently than the mature pericytes. Same results were obtained in murine skeletal muscle. Interestingly, we confirmed this pericyte-heterogeneity in human adipose, cardiac, and skeletal muscle tissue. Ongoing experiments both in mouse and human will address the therapeutic potential of the novel pericyte-progenitors we described.

#35

Pannexin1 regulates neurite development via a novel protein-protein interaction with Collapsin response mediator protein 2

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Neurite formation requires precise remodelling of cytoskeletal proteins. As an ion and metabolite channel enriched in the CNS, Pannexin1 (Panx1) has been demonstrated to be involved in synaptic plasticity as well as learning and memory. Previous work in our lab has shown it negatively regulates neurite outgrowth and physically associates with actin. Here we further demonstrate that blocking Panx1 with probenecid favors microtubule stabilization. In order to shed light on the underlying mechanism, unbiased proteomic analysis was carried out and revealed a well-known microtubule-associated protein-collapsin response mediator protein 2 (Crmp2) as a Panx1 interacting protein. Capitalizing on immunoprecipitation as well as Proximity ligation assay (PLA), this novel interaction was confirmed *in vivo* and *in vitro.* Additionally, *in vitro* binding assays between purified proteins not only suggested this interaction was direct, but also limited the binding area to Panx1 C-terminus. Therefore, we hypothesized that Panx1 might regulate neurite development via this novel interplay with Crmp2.

To test our hypothesis, we next treated Neuro2a cells with probenecid and discovered that inhibiting Panx1 markedly reduced the Panx1/Crmp2 interaction, suggesting Crmp2 was “released” from Panx1/Crmp2 protein complex. Moreover, Crmp2 concentrated on the distal tips of neurites after probenecid treatment, where microtubules are reported to be more dynamic compared to in neurite shaft. Intriguingly, the Panx1 blocker also decreased Crmp2 phosphorylation at serine 522, resulting more un-phosphorylated Crmp2, which is recognized as an active molecule facilitating microtubule assembly through binding to alpha- and beta- tubulin heterodimers.

In summary, these results reveal a new protein-protein interaction between Panx1 and Crmp2. Panx1 effectively ‘keeps the brakes’ on neurite outgrowth by physically sequestering Crmp2. Loss of Panx1 function during development might remove this “brake” and release more “active” Crmp2, thereby promoting neuritogenesis. Our investigations also have important implication regarding the role of Panx1 in regulating the cytoskeleton in the context of neuroplasticity.

#36

A xeno-free culture system for efficient derivation and amplification of human endothelial colony-forming cells from umbilical cord blood

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Endothelial colony-forming cells (ECFCs) have been identified as endothelial progenitor cells with robust proliferative potential *in vitro* and vasculogenic properties *in vivo*. Thus, ECFCs have emerged as a target for regenerative therapies. However, potential therapeutic use of ECFCs is compromised by low frequency in umbilical cord blood (UCB) so samples must be expanded *in vitro* prior to use. Current formulations for expanding ECFCs *in vitro* contain fetal bovine serum (FBS), making them inappropriate for clinical use. To minimize the risks associated with exposure to animal serum, new culture media are required. Here we compared ECFCs derived and expanded from primary UCB in EC Cult™-XF ECFC medium, a novel xeno-free (XF) culture system that does not contain FBS, to ECFCs generated in serum-containing (SC) medium. Colonies generated by UCB were counted on day 10, and then expanded by passaging the cells in the medium in which they were derived. The hierarchy of ECFCs was evaluated using clonogenic assays. Expression of CD144, CD31 and CD45 as well as acetylated LDL (Ac-LDL) uptake was tested at P5. The functional capacity of ECFCs to mediate vascular repair was evaluated in a mouse model of oxygen-induced retinopathy (OIR). XF medium supported an equivalent frequency and expansion of ECFC colonies as SC medium. EC-Cult™-XF ECFC supported the entire hierarchy of ECFCs, with larger colonies forming in XF than in SC medium. More than 97% of cells cultured in either media were CD144+CD31+, CD45- and incorporated Ac-LDL. In the OIR model, injection of ECFCs derived in EC Cult™-XF medium decreased the area of vascular obliteration and neovascular tufts. In summary, ECFCs can be efficiently derived and expanded in xeno-free EC Cult™-XF medium, facilitating their use in translational research.

#37

A novel animal component-free culture medium for the enhancement of human mesenchymal progenitor cell expansion

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MesenCult™-ACF-Plus is an improved animal component-free (ACF) culture medium for the derivation and expansion of mesenchymal progenitor cells (MPCs) from primary human bone marrow mononuclear cells (BMMC). This new medium is formulated to further enhance the expansion of MPCs obtained in a first generation medium, MesenCult™-ACF. To compare the performance of MesenCult™-ACF- Plus with MesenCult™-ACF, MPCs derived from primary BMMC were plated at 1-5x10^4 cells/cm^2 in each formulation. Clonogenic growth was evaluated using the Colony-Forming Unit-Fibroblast (CFU-F) assay. To evaluate the expansion of MPC, BMMC were plated initially at 3–5 x 10^4 cells/cm^2, and for subsequent subculture MPCs were plated at 1.5–3x10^3cells/cm^2 in each medium. The proliferative potential of MPCs was determined by counting the number of cells at each passage (P) up to P6. The generation of total CFU-F per 10^6 BMMC was comparable in the two media (71 ± 26 versus 69 ± 27 [mean ± SEM; n=6] in MesenCult™-ACF- Plus and MesenCult™-ACF, respectively). The average fold-expansion of MPCs at each subculture was significantly higher in MesenCult™-ACF-Plus medium (11.0 ± 1.3 mean ± SEM; n=4) than in MesenCult™-ACF (7.7 ± 0.4 mean ± SEM; n=4; $p<0.05$). Indeed, the expansion of MPCs in this new MesenCult™-ACF- Plus medium is as robust as that obtained in MesenCult™-XF (11.0 ± 1.3 versus 11.8 ± 0.8 [mean ± SEM; n=4]; $p>0.05$ in MesenCult™-ACF- Plus and MesenCult™-XF, respectively). The MPCs generated in MesenCult™-ACF-Plus can be cryopreserved in MesenCult™-ACF Freezing Medium at any passage with high cell viability and recovery upon thawing (92.2% ± 2.9 and 74.8% ± 3.7 [mean ± SEM]; n=3, viability and recovery, respectively). In summary, MesenCult™-ACF- Plus supports robust MPC expansion and together with the use of MesenCult™-ACF Freezing Medium provides a complete ACF culture workflow for derivation, expansion and cryopreservation of these cells. Moreover, the MPCs cultured in MesenCult™-ACF-Plus differentiated robustly under the appropriate conditions into adipocytes, osteogenic cells and chondrocytes. Experiments to characterize MPCs cultured in MesenCult™-ACF- Plus medium from other (non-BM) tissue types are underway. The performance of MesenCult™-ACF-Plus medium is superior to that of the existing MesenCult™-ACF and equivalent to MesenCult™-XF.

#38

Engineering early human development

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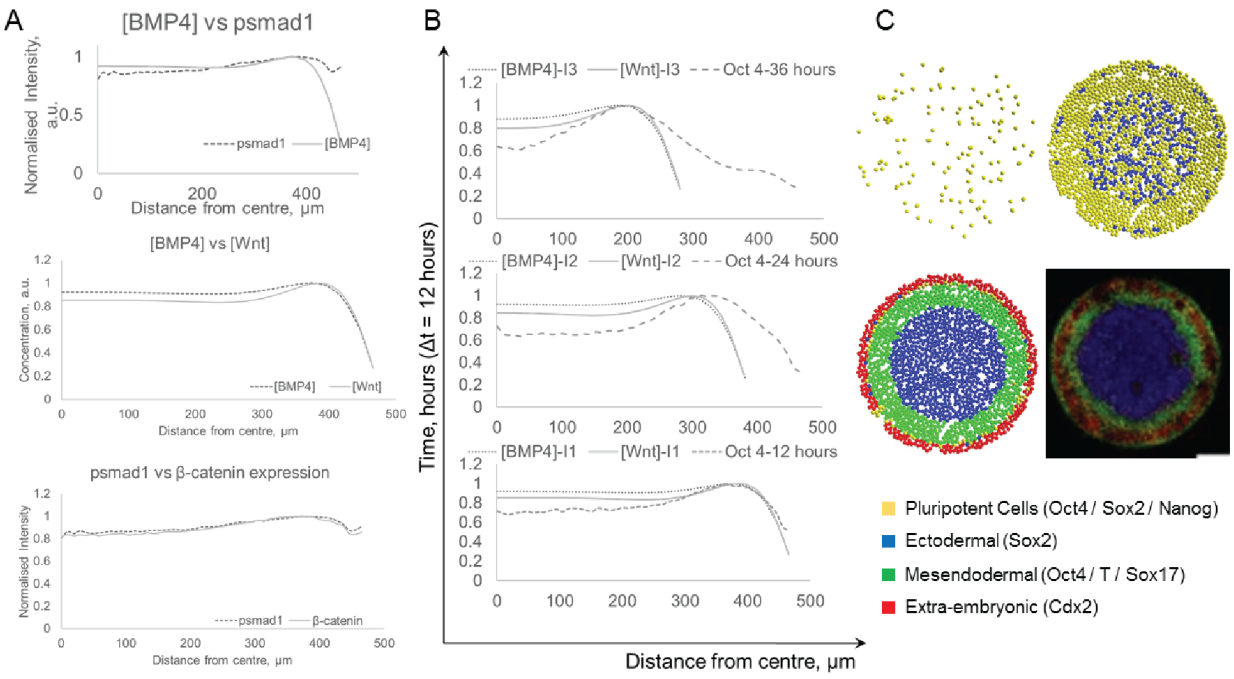
Understanding the interplay between gene regulatory networks (GRN) and a dynamically evolving cellular microenvironment is crucial to reliably engineer synthetic tissues from human pluripotent stem cells (hPSCs). We employ embryonic organoids, i.e. hPSC colonies cultured on 2D micropatterns, which upon stimulation with BMP4 and Nodal show spatial arrangement reminiscent of a gastrulating embryo, to understand how the genetic circuitry of hPSCs integrates environmental information to lead to functional heterogeneity.

We employed embryonic organoids to observe the spatiotemporal evolution of pluripotency (OCT4), ectodermal (SOX2), mesodermal (Brachyury), and extra-embryonic (CDX2) markers. Furthermore, pSMAD1 and β-catenin expression profiles, activated downstream of BMP4 & Wnt3a, respectively, were also recorded. To quantify how the various signalling pathways interacted with each other as well as the cells' genetic circuity, we developed GRN inference strategies to predict the GRNs controlling cell responses to exogenous cues and integrated these Boolean networks into an agent-based framework, which itself was coupled with a novel four-component reaction-diffusion model capturing interactions between BMP4-NOGGIN & Wnt3a-DKK1.

The computational model recapitulated the empirical pSMAD1 and β-catenin profiles (**A**), and additionally showed how morphogen reaction-kinetics create dynamic boundary conditions that govern the expression of the germ-layer markers in embryonic organoids over time (**B**). The fully integrated computational model with a preliminary GRN embedded with an agent-based model, which itself was localised within a dynamic environment, allowed us to capture the spatiotemporal roadmap of how the GRNs interact with various signalling pathways to result in spatially distinct patterns in functionally homogeneous hPSC colonies (**C**).

This enabled us to develop a detailed roadmap of events that lead to the emergence of spatially distinct germ-layer markers in embryonic organoids, and demonstrate for the first time how dynamically evolving boundary conditions within biological systems control developmental fate.



A: Computational concentration profiles agree with empirical pSMAD and β-catenin expression profiles. B. Temporal changes in [BMP4] & [Wnt3a] profiles predict OCT4 expression over 36 hours. C. Integrated model captures spatiotemporal evolution of patterns.

#39

Brain organoids: A transformative, new research tool for neuroscience research

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Brain organoids, in particular, are a significant improvement over existing investigative tools that are used in neuroscience research. In drug discovery, for example, current two-dimensional *in vitro* cultures comprising cortical neurons do not incorporate the blood-blood barrier (BBB). As a result, the diffusion of the lead compound across the BBB, which has long been the Achilles heel of therapies targeting the brain, is never actually evaluated until the molecules are tested in animal models of the disease. Here too, the tissue architecture within rodent brains that are presently utilized for *in vivo* testing is quite dissimilar to that of the human brain. Unsurprisingly, results from pre-clinical testing – regardless of the therapeutic hypothesis – do not translate well to the clinic, and nearly all drug candidates targeting neurological diseases fail when tested in real patients on account of poor safety and/or efficacy. Beyond drug discovery, the use of brain organoids could also be extended to investigating early brain development and identifying the mechanisms that elicit neurodegeneration. **In this review we outlined the state-of-the-art regarding fabrication and use of brain organoids in drug development and medical research, highlight key scientific challenges that would need to be addressed in order to enhance their relevance as a model tissue, and offer potential solutions to these challenges, including incorporation of emerging biomedical technologies such as bioprinting.** And in particular we discussed:

1. Summary of the materials and methods used for fabrication of brain organoids, with a special emphasis on the methods developed by Lancaster *et al.*[1,2] and Paşca *et al.*[3]
2. Comparison between the neurobiology of brain organoids and brain tissue
3. Highlights of current and emerging applications for brain organoids
4. Contrast between brain organoids with other investigative platforms such as the brain-on-chip
5. Overview of current limitations and knowledge gaps in the technology and a listing of key scientific challenges that would need to be addressed to ensure greater physiological relevance
6. Potential solutions to aforementioned scientific challenges, including use of emerging technologies such as bioprinting and microfluidics

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#40

Spatially organized gene expression heterogeneity in mouse pluripotent stem cells

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The survival of any given cell population often depends on population heterogeneity. For example, populations of unicellular organisms adapt to changing environments by committing subpopulations to different fates, a probabilistic response which allows the population to survive a larger range of environmental changes than a homogeneous cell population. Likewise, developmental gene expression heterogeneity is instrumental in allowing single cells to generate multicellular organisms. This heterogeneity is often described as stochastic, but recent studies have revealed hidden variables explaining much of the variation in developmental cell fate acquisition. Identifying such variables has also shed light on the deterministic origins of heterogeneity *in vitro*. Specifically, the cellular microenvironment is critical in directing cell fate acquisition, as observed during differentiation in micropatterned human pluripotent stem cell (PSC) colonies.

Whether microenvironmental variation impacts heterogeneous expression during maintenance of pluripotency remains to be investigated. Studies on this topic are critical not only for developing an understanding of fundamental cell fate acquisition principles, but also because the probabilistic differentiation capabilities of PSCs severely limit their effectiveness and safety as therapeutic agents. In culture, mouse PSCs can be sustained in either a relatively homogeneous naive or primed state, or as a heterogeneous combination of these two states. Coupled with our vast knowledge of mouse development, mouse PSCs offer a powerful model to study the impact of microenvironmental variations on gene expression.

We have developed an analytical software pipeline to automate the classification of cell colonies and the quantification of spatially organized gene expression in our high-throughput micropatterning immunocytochemistry platform. Using this platform to quantify pluripotency markers in human PSCs, we observed that expression patterns clustered in three categories: evenly throughout the colony, decreasing gradually towards the edge, or highly expressed as a ring. We are currently employing this platform to investigate the effect of microenvironmental variation on the observed gene expression heterogeneity in mouse PSCs. Encouragingly, preliminary results show radial organization of pluripotency and differentiation markers within colonies cultured in pluripotent conditions. Specifically, we observe spatial organization of Brachyury in mouse PSC colonies of different shapes and sizes in maintenance conditions containing serum and LIF.

#41

Novel method of cardiomyocyte differentiation of induced pluripotent stem cells using co-culture with induced pluripotent stem cell-derived cardiomyocytes

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One of the strategies of generating cardiomyocytes from stem cells is by co-culture with an appropriate inducer. This approach stem from the logical reasoning that one can overcome the inherent limitation of precisely recapitulating the biochemical signaling events associated with cardiac organogenesis by handing the task over to already differentiated cardiomyocytes.

The first reported success of creating cardiomyocytes from human pluripotent stem cells via co-culture induction came from Mummery's group. Through their groundbreaking works, they showed that co-culture of human embryonic stem (hES) cells with mouse visceral-endoderm-like (VE-like) cells initiated differentiation into beating cells. Similarly, when Rudy-Reil's group co-cultured murine embryonic stem cells with a bilayer of avian precardiac endoderm / mesoderm, the number of contractile embryoid bodies was significantly increased compared to cells cultured alone. More recently, Dong-Bo Ou's team demonstrated that the long-term differentiation of mouse embryonic stem cells (ESCs) into cardiomyocytes (CMs) proved more efficient when co-cultured with mouse neonatal cardiomyocytes compared to sole treatment with ascorbic acid to induce cardiac differentiation.

Noticeably lacking from existing literature however is a co-culture inductive platform utilizing stem cell-derived cardiomyocytes as a stimulatory source for cardiac reprogramming (of stem cells or otherwise). We addressed this knowledge gap by investigating the potential of a cardiac differentiation method based exclusively on co-culture with previously differentiated iCMs as an inducer.

Our proof-of-principle experiments revealed that previously differentiated iCMs co-cultivated with iPS cells constituted a sufficient stimulatory system to induce cardiac differentiation – as determined by the exhibition of spontaneous self-contractions, expression of cardiac specific markers, and structural organization of sarcomeres. This process was achieved without the exogenous addition of pathway inhibitors and morphogens, suggesting that 'older' iCMs serves as an adequate stimulatory source capable of recapitulating the necessary culture environment for cardiac differentiation.

#42

Boolean network modeling of T cell development predicts heterogeneous single cell transcriptional trajectories

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The T cell development program drives blood progenitors toward T lineage commitment using dense networks of genes and proteins that respond to environmental signals. Although existing static models of these networks identify the major players and their pairwise interactions, they provide limited explanatory insight into the multi-stage dynamics of T cell specification. In this study, we use a computational approach based on Boolean networks (BNs) to simulate aspects of the T cell development program *in silico* and explore the dynamic response of progenitor cells under normal and perturbed conditions. A BN representation of the regulation of each gene in the network using AND/OR logic was constructed from microarray data and previous literature. By using asynchronous updates to simulate this BN, we mapped the transcriptional state space that developing T cells can traverse under various combinations of environmental inputs (such as Notch and Interleukin-7 signaling). This state space coincides with single cell qRT-PCR observations, and steady states within the space resemble known stages of T cell development. Notably, the BN model suggests the T cell development program permits multiple independent transcriptional trajectories toward T lineage commitment that involve different transient cell states. Furthermore, our analysis identifies signaling cues and genetic perturbations that may select for particular differentiation trajectories or arrest development at specific stages. BN modeling presents a powerful advance over previous static models for exploring routes through the transcriptional space involved in T cell development and suggests new opportunities for improved *in vitro* T cell differentiation protocols.

#43

Characterizing voltage and calcium dynamics of atrial-like cardiomyocytes derived from human induced pluripotent stem cells (hiPSCs) using optical mapping assay

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Atrial fibrillation (AF) is the most common cardiac arrhythmia worldwide with an expected increase in prevalence over the coming decades. Thus, it is imperative to find novel treatments for better management of the disease. However, current preclinical assays such as heterologous expression and animal models do not recapitulate the entirety of human cardiac physiology. As such, the advent of hiPSC-derived cardiomyocytes (hiPSC-CMs) have provided a more robust physiological system to assess drug effects *in vitro*. Additionally, the ability to generate atrial- and ventricular-like hiPSC-CMs has given the opportunity for preclinical screening of AF treatments. An important aspect of antiarrhythmic compounds for AF treatment lies in the ability to have atrial-selective effects with minimal risk of ventricular proarrhythmia. Nevertheless, current drug screening assay systems capable of characterizing atrial effects using hiPSC-CMs are limited. Here, we demonstrate an approach using atrial-like cardiomyocytes derived from hiPSCs coupled with sophisticated optical mapping for drug screening of atrial-selective compounds *in vitro.*

We first differentiated atrial- and ventricular like hiPSC-CMs using two distinct protocols. The voltage and calcium transients (Ca^{2+}T) of the two hiPSC-CM subtypes were then characterized by optical mapping using potentiometric dye RH-237 and Ca^{2+} fluorescence probe Rhod-2AM. Furthermore, both hiPSC-CMs subtypes were electrically paced to measure the effective refractory period (ERP) and to interrogate rate-dependent properties of drug response.

Atrial-like hiPSC-CMs have shorter action potential (AP) durations at 30% and 50% (APD_{30} and APD_{50}) than hiPSC-derived ventricular CMs, while possessing a triangulated AP morphology. Interestingly, Ca^{2+} transients are comparable between the two cell types. Additionally, the atrial-selective drug vernakalant increases the ERP of atrial-like hiPSC-CMs in a dose-dependent manner.

Overall, the ability to differentiate cardiac subtype specific hiPSC-CMs coupled with optical mapping assay constitute a comprehensive *in vitro* assay system for future drug development and personalized medical treatment of AF.

#44

Delineating definitive endoderm organogenesis at single-cell resolution

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During gastrulation, the three germ layers of the embryo are established: the ectoderm, the mesoderm and the definitive endoderm (DE). At embryonic day 8.5 (E8.5), patterning of the DE begins to define regions in the epithelial sheet where the future organs will form. Considering that these tissues are major sites of health problems, including congenital defects, cancers, and organ malfunctions, we know surprisingly little about the early embryonic development of the DE. The mechanisms how cell fate or organ domains are determined in DE are poorly understood.

One of the primary hindrances in understanding gut organogenesis is a lack of information on the genes involved in the formation, specification, and differentiation of the DE. To address this, we undertook a genome-wide transcriptome analysis of early endoderm in the mouse embryo and identified several novel genes that have unique expression domains in the DE, including Nephrocan (NEPN) which specifically labels midgut. To further delineate gene expression in the mouse DE at single cell resolution, we employed 10x Chromium high-throughput single-cell 3' RNA-seq to generate libraries of 9252 cells from E8.5 (15ss) endoderm. To visualize cell diversity, we use Phenograph, a previously developed graph-based clustering algorithm that is based on Wishbone and MAGIC. Further, we employed MAST, GSEA, and Wishbone to explore differential gene expression within cell types. Interestingly, we unveiled a subset of NEPN decedent cells that are connecting DE with visceral endoderm (VE) using E8.5 scRNAseq database and lineage tracing. Overall, we have generated the first landscape of DE organogenesis in the mouse and provided insight into the dynamic gene expression patterns. Our study will benefit pluripotent stem cell differentiation into DE derivatives in the future.

#45

An efficient method for generation of bi-allelic null mutant mouse embryonic stem cells and its application for investigating epigenetic modifiers

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Mouse embryonic stem (ES) cells are a popular model system to study biological processes, though uncovering recessive phenotypes requires inactivating both alleles. Building upon resources from the International Knockout Mouse Consortium (IKMC), we developed a targeting vector for second allele inactivation in conditional-ready IKMC 'knockout-first' ES cell lines. We applied our technology to several epigenetic regulators, recovering bi-allelic targeted clones with a high efficiency of 60%, and used Flp recombinase to restore expression in two null cell lines (Jarid2 and Cbx1) to demonstrate how our system confirms causality through mutant phenotype reversion. We designed our strategy to select against re-targeting the 'knockout-first' allele and identify essential genes in ES cells, including the histone methyltransferase Setdb1. For confirmation, we exploited the flexibility of our system, enabling tamoxifen inducible conditional gene ablation while controlling for genetic background and tamoxifen effects. Setdb1 ablated ES cells exhibit severe growth inhibition, which is not rescued by exogenous Nanog expression or culturing in naive pluripotency '2i' media, suggesting that the self-renewal defect is mediated through pluripotency network independent pathways. Our strategy to generate null mutant mouse ES cells is applicable to thousands of genes and repurposes existing IKMC Intermediate Vectors.

#46

Transcriptional profiling of adult hair follicle mesenchyme reveals R-spondin and Hic1 as novel regulators of dermal stem cell fate and function

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Hair follicles (HFs) contain specialized mesenchymal (dermal papilla) cells that drive continuous tissue regeneration by providing inductive signals to HF epithelial cells. Loss of DP cells prevents HF regeneration.

We recently identified a hair follicle dermal stem cell (hfDSC) that functions to maintain the HF mesenchyme, consisting of the inductive dermal papilla (DP) and dermal sheath (DS) that surrounds the proximal HF. Understanding the mesenchymal signals that regulate hfDSC function and instruct epithelial function during HF regeneration will be essential for developing therapeutic interventions aimed at restoring skin function following aging, injury or disease.

To probe the hfDSC cell lineage for genes responsible for HF regeneration, we performed prospective isolation and RNAseq, and established genetic signatures for each mesenchymal compartment (DP, DS, hfDSCs). The Wnt potentiator Rspondin-2/3 showed an 8-fold differential upregulation within the DP relative to all other compartments. FACS-isolated hfDSCs treated *in vitro* with Rspo-2/3 or in combination with a Wnt-agonist resulted in robust increase in colony size and number compared to control. Intradermal injection of Rspo-2/3 into mouse back skin during telogen (rest) resulted in robust induction of HF regeneration. We also found that the tumor suppressor hypermethylated-in-cancer 1 (Hic-1) was uniquely expressed within hfDSCs. Fate mapping using Hic1CreER^{T2}:ROSA^{Tomato} mice revealed that Hic1 marks at least a subset of bipotent adult hfDSCs that persist over multiple regenerative cycles. Inducible deletion of Hic1 in hfDSCs or Rspo3 in DP cells was also performed. Finally, experiments pertaining to translation of these results to adult human scalp biology are described.

#47

Resolving the endodermal to hepatic switch at the single-cell level

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Hepatocytes are the major functional cell type in the adult liver and perform many roles including blood detoxification, bile production, lipid metabolism, and liver regeneration. These cells are derived from the endoderm, one of the three primary germ layers in the early embryo that gives rise to the gastrointestinal and respiratory tracts. At embryonic day (E)9, signals from the septum transversum mesenchyme specify a subset of these endodermal cells as liver progenitors and cause them to undergo an EMT-like process, where they delaminate from the endoderm and migrate into the mesenchyme, forming the liver bud. Around the same time, haematopoietic stem cells colonise the liver bud, where it remains the major site of haematopoiesis until around E16. Genetic models have shown that the perturbation of embryonic liver cell-types, including mesencyhmal, endodermal, or haematopoietic cells results in impaired hepatocyte development and function, but the mechanisms remain poorly understood.

To resolve the contribution of these different cell types during the endodermal to hepatic switch, we have employed 10X Chromium high-throughput single-cell 3' RNA sequencing to generate libraries of 9252 cells from the E8.5 endoderm and 2643 cells from E9.5 liver buds. We used Phenograph, a graph-based clustering method based on Wishbone developmental trajectory positioning and MAGIC data imputation algorithms. We have also performed differential gene expression analysis and pseudotime trajectory estimation using MAST and Monocle, respectively. These data reveal the cellular heterogeneity present during the earliest stages of liver development and point to possible cellular interactions guiding the endodermal to hepatic switch.

#48

HNF4a mediated epigenetic regulation of liver-specific genes requires its interaction with TET3

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Transcription factors (TFs) are key components of gene regulation and their binding to *cis* regulatory regions can establish the expression status of genes, leading to the specification of cell fate. Hepatocyte nuclear factor 4 alpha (HNF4a), a liver enriched transcription factor, plays an important role in specification of hepatic progenitor cells by regulating a network of TFs that controls the onset of hepatocyte cell fate. Our HNF4a ChIP-seq, MeDIP-seq hMeDIP-seq data for hepatocytes showed that HNF4a primarily binds to enhancer regions with a very low level of DNA methylation and high hydroxymethylation. Further, we confirmed that HNF4A physically interacts with TET3 in hepatocytes and HepG2 cells to hydroxymethylate these enhancers. Knockdown of HNF4a or TET3 results in a significant decrease in global hydroxymethylation in HepG2 cells, indicating its role in epigenetically shaping enhancers by retaining transcription permissive marks at enhancers required to maintain expression of liver specific genes. Furthermore, we found that HNF4A induces TET3 expression via direct binding to its *cis*-regulatory elements. Based on these observations, we confirmed that HNF4a regulates liver specific gene expression by its interactions with TET3 DNA demethylases, to protect distal gene regulatory sites from the transcriptional repressive influences of DNA methylation during liver development. Overall, our findings present a novel mechanism of HN4a mediated genes regulation in liver.

#49

Single-cell characterization of a developmentally conserved phenotype of primitive human hematopoietic cells

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Clinical gene therapy studies have demonstrated the ability of individually-tracked transplanted hematopoietic stem cells (HSCs) to sustain the production of mature blood cells for many years. Interestingly, the lineage output and clone size of each HSC has been found to be unique and stable. Clonal tracking of the progeny of mouse HSCs has also demonstrated extensive, intrinsically determined, but differently regulated, heterogeneity in HSC self-renewal and differentiation activities, with changes in the distributions of HSCs with these different properties during development and aging. Analogous studies of HSCs from young and old human sources should provide insight into mechanisms that contribute to an increased age-associated incidence of oligoclonal hematopoiesis and acute leukemia. To move towards the isolation and biological characterization of human HSCs in adult bone marrow for comparison to their counterparts in human cord blood, we investigated properties of Lin-CD34+38-45RA-90+49f+ ("49f") cells, a phenotype highly enriched for HSCs, from both sources. Paired analyses of the molecular and functional properties of 49f cord blood cells revealed a CD33+90^{hi} subset enriched for durable outputs of mature blood cells both in 8-week stroma-containing cultures, and in serially transplanted immunodeficient mice. Matched analysis of 49f bone marrow cells from normal adult donors showed a similar correlation of prolonged proliferative ability *in vitro* with elevated CD90 on the input cells but ubiquitous expression of CD33 and an overall reduced frequency of 49f cells with outputs for 8 weeks *in vitro*. In serum-free medium supplemented with a highly stimulatory growth factor combination, adult 49f bone marrow cells took a similar time as cord blood 49f cells to complete a first cell cycle, but were slower in completing subsequent divisions, and hence divided less overall in the 7-day tracking period. These results provide definitive evidence of both qualitative and quantitative changes in the 49f subset of cells isolated from normal humans at different stages of development. They also serve to emphasize the importance of parallel single-cell molecular approaches to elucidate the responsible mechanisms.

#50

Improving regeneration and decreasing damage in Muscular Dystrophy: the role of cholesterol

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Purpose: The pleiotropic, non-lipid lowering effects of statins were recently shown to attenuate muscle wasting in Muscular Dystrophy (MD). In the mdx mouse model of Duchenne MD, simvastatin ameliorated intramuscular inflammation and fibrosis, leading to improved muscle function and heightened regeneration. Our team has shown that mdx and Dysferlin$^{-/-}$ mice, two notoriously mild models of MD, exhibit severe muscle wasting and loss of ambulation when their plasma cholesterol profile is altered. Hence, we hypothesized that lowering of cholesterol, rather than pleiotropism, is the true mechanism of simvastatin in MD and a pharmacological target.

Methods: To test the contribution of cholesterol to MD severity and evaluate the feasibility of modulating cholesterol in MD, the current study explores the effect of medications typically used in human dyslipidemia in two mouse models of MD (Dysferlin$^{-/-}$ and mdx) with a humanized plasma lipoprotein profile; this was done by inactivating their Apolipoprotein E (ApoE) gene (a common model of atherogenesis), as well as either western diet (triglyceride-rich (TG), 0.2% cholesterol) or high (2%) cholesterol chow-based diet supplementation. Treatment spanned 2-11 months (mo.) for Dysf$^{-/-}$/ApoE$^{-/-}$, and 2-7mo. for mdx/ApoE$^{-/-}$ cohorts and their appropriate controls. Muscles stained with Masson's Trichrome were used to assess fat/collagen deposition and regeneration.

Results: Compared to Dysf$^{-/-}$/ApoE$^{-/-}$ mice on chow, a TG/0.2% cholesterol containing diet increased muscle wasting and severe fibro-fatty infiltration in key muscles, leading to complete ambulatory dysfunction in ~40% of groups by 11mo. This effect was rapidly accelerated to 4-5mo. of age when cholesterol was increased to 2%. Similar effects of elevated lipid levels were observed in the muscles of mdx/ApoE$^{-/-}$ (TG/0.2%) by 7mo. of age. Strikingly, treatment with cholesterol-lowering agents reversed the early signs of gait abnormality and prevented the loss of ambulation in our 0.2% cholesterol fed Dysf$^{-/-}$/ApoE$^{-/-}$ mice. Moreover, treatment completely prevented the accumulation of intra/intermuscular lipids and improved regenerative events in the triceps and quadriceps muscle groups, as well as triceps and gastrocnemius muscles of mdx/ApoE$^{-/-}$ mice.

Conclusions: Our data show that cholesterol causes severe muscle damage and loss of ambulation in MD, an effect likely attributable to heightened muscle damage and poor regeneration.

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