1. Full Optogenetic Control of Human Cardiomyocytes and Engineered Heart Muscle

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The ability to use light to stimulate mammalian cells has significantly augmented our understanding of electrically excitable tissues in health and disease, paving the way toward various novel applications in the research and therapeutic fields. Here, we demonstrate full optogenetic control of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). We simultaneously introduced channelrhodopsin-2 (ChR2) and a third-generation halorhodopsin (NpHR3) into hiPSC via a polycistronic lentiviral vector. Via directed differentiation, we created opsin-expressing cardiomyocytes. To quantify the impact of photostimulation on these cells, we assessed evoked electrical and mechanical signals. With the application of blue (470 nm) and yellow (580 nm) light, we show both activation and inhibition of cardiomyocyte contractions, respectively. To illustrate the utility of our system, we have synchronized our light-sensitive cardiomyocytes with discarded primary human heart tissue, in vitro. In addition, we have created three-dimensional optogenetic engineered heart muscle (o-EHM) that can be activated and inhibited with light; this offers a way to immediately and orthogonally synchronize this muscle with native heart rhythms. Our system provides insight into whether hiPSC-CM have the potential to be synchronized to recipient hearts in vivo upon therapeutic delivery, either as individual cardiomyocytes or as a component of engineered muscle.

2. New Non-Invasive Method for Determining Blood Pressure Through the Hind Leg of Unanaesthesized Rats

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Invasive method of determining blood pressure has been the commonly used method in animal model of hypertension study. While majorly used non invasive blood pressure monitoring devices are very costly and unaffordable by researchers from developing or under developed countries including Nigeria. In our study, we designed a new, cheap non invasive method for determining blood pressure through hind leg of rats by using ambulatory blood pressure monitoring device (CONTEC 08A) with small cuff (3-5 cm) for rats. Ten male Wistar rats of 182-240 g body weight were randomly assigned to two groups (n=5/group). A group served as control (physiological normal rats), the second group was administered single dose 2mg/kg i.p dexamethasone supplemented with 4% table salt (NaCl) as drinking water to induce hypertension. Blood pressure was measured ten times in each rats of the two groups at baseline (day 0) and after 5 days. Reproducibility (Sw) was calculated in each group. CONTEC 08A yielded good reproducibility in both hypertensive (SBP, Sw = 6 mm Hg, DBP, Sw = 10 mm Hg) and non hypertensive rats (SBP, Sw = 3 mm Hg, DBP, Sw = 6 mm Hg). Better reproducibility was obtained in non hypertensive rats. Consistency in data obtained showed that non invasive blood pressure monitoring from the hind leg of rats using CONTEC 08A device with small cuff is effective, and recommendable for use in rat model study of hypertension.

3. Generalized Equation of the Pulmonary Circulation, Implications for Epidemiological and Longitudinal Studies in Pulmonary Hypertension Ccross WHO Groups

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Background: The strong linear relation between mean (MPAP) and systolic (SPAP) pulmonary arterial pressure (e.g. SPAP = 1.62 x MPAP) has been mainly reported in pre-capillary pulmonary hypertension (PH). This study sought to assess the MPAP-SPAP relation across WHO PH groups, accounting for pulmonary artery wedge pressure (PAWP), heart rate and age.

Methods: An allometric equation relating MPAP and SPAP was developed in 1,135 patients with pulmonary arterial hypertension (PAH), advanced lung disease, chronic thromboembolic PH, or advanced heart failure. The equation was validated in 60,885 patients from the UNOS database referred for heart and/or lung transplant. The MPAP/SPAP longitudinal stability was assessed in PAH.

Results: The equation obtained was SPAP = 1.39 x MPAP x PAWP-0.07 x (60/heart rate)0.12 x age0.08 ($p<0.001$). It was validated in the UNOS cohort ($R2=0.93$, $p<0.001$), regardless of the type of organ(s) patients were listed for (mean bias [-1.96SD;1.96SD] was 0.94 [-8.00;9.88] for heart, 1.34 [-7.81;10.49] for lung and 0.25 [-16.74;17.24]mmHg for heart-lung recipients). Thresholds of SPAP for MPAP=25mmHg were lower in patients with higher PAWP (35.5 mmHg vs. 41.5 mmHg). In 186 patients with PAH, the normalized MPAP/SPAP was stable over time (0.63±0.03 at baseline and follow-up catheterization, $p=0.43$) with lower coefficient of variation than the resistance-compliance time product (8% vs. 25%).

Conclusions: This study demonstrates the impact of PAWP, and in a lesser extent heart rate and age on the MPAP-SPAP relationship, leading to slightly different SPAP thresholds for PH diagnosis across WHO groups, which is relevant for echocardiography-based epidemiological studies.

4. Circulating Immune Profile of Aging: a Translational Study on the Role of Growth Differentiation Factor-15 and Monokine induced by Interferon Gamma

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Introduction: While inflammation is frequently found in elderly individuals, it remains controversial whether healthy aging is associated with an increase in pro-inflammatory markers.

Objective: To determine the immune profile associated with aging and identify the endothelial effect of these molecules.

Methods: The study included a first derivation cohort of 102 healthy individuals (age 20-90 yo, mean age 57.4±18.3 years, 55% of females) free of cardiovascular disease, inflammatory condition or cancer. Targeted immune profiling (48-plex Luminex), C-reactive protein, Growth Differentiation Factor-15 (GDF-15), galactin-3 and metabolic profiling with insulin and comprehensive lipid panel was performed. For validation, we profiled an additional cohort of 78 less healthy individuals and applied an in-vitro endothelial cell validation model investigating the effects of immune molecules on aging stressors.

Results: In the derivation cohort, GDF-15 and Monokine induced by Interferon Gamma (MIG also known as CXCL9) were strongly associated with the cardiovascular parameters: pulse wave velocity (PWV) is a marker of arterial stiffness and mitral lateral e' is a marker of myocardial relaxation.. These cytokines were the most correlated with age ($R^2=0.73$ and $R^2=0.59$, for PWV and e', respectively, $p<0.001$), a finding which was confirmed in the validation cohort.

In-vitro treatment with GDF-15 and CXCL9 attenuated the endothelial dysfunction phenotype (i.e. decrease in eNOS and increase in ICAM-1 mRNA endothelial expression) after exposure to IL-1B and oxidative stress.

Conclusions: GDF-15 and CXCL9 are strongly correlated with age and have modulating effects on inflammasome or oxidative stress mediated endothelial cells injury.

5. Alternatively Activated Macrophage Exosomes: New Mediators to Control Hematopoiesis & Atherosclerosis

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Introduction: Macrophages have been shown to produce exosomes that can travel in the circulation and be detected in atherosclerotic lesions. But whether they impact lesion dynamics is currently unknown. We investigated the potential therapeutic value of exosomes produced by alternatively activated M2-like macrophages on controlling inflammation and atherosclerosis.

Methods: Using cushioned-density gradient ultracentrifugation, exosomes were isolated from the cell culture medium of naïve bone marrow derived macrophages (BMDM-exo) and BMDM exposed to interleukin 4 (IL-4) to polarize them into an M2 phenotype (BMDM-IL-4-exo).

Results: Our results demonstrated that BMDM-IL-4-exo displayed anti-inflammatory properties when tested in vitro. BMDM-IL-4-exo reduced Tnf and Il1b mRNA expression in recipient cultured BMDM and enhanced mitochondrial oxidative metabolism that further increased their M2 differentiation. Treatment of Apoe–/– mice fed a western diet during a period of 4 weeks with BMDM-IL-4-exo reduced the number of myeloid cells in the circulation by controlling excessive hematopoiesis in the bone marrow. Furthermore, BMDM-IL-4-exo had a profound impact on reducing the necrotic core area and the number of macrophages in aortic root lesions. To identify molecular mechanisms responsible for these protective effects, we performed unbiased RNA sequencing of BMDM exosomes which revealed that BMDM-IL-4-exo are enriched in candidate microRNA as compared to control BMDM-exo. Gene editing to selectively knock down each one of these microRNA in IL-4 polarized macrophages resulted in exosomes that were less effective in reducing NF-κB signaling and levels of TNFa in recipient macrophages.

Conclusions: Our findings reveal that M2-like macrophage exosomes are potent anti-inflammatory mediators through the delivery of select microRNA to recipient cells. Therefore, engineering macrophage exosomes with microRNA targeting inflammatory pathways could provide a therapeutic approach to promote the resolution of inflammation in atherosclerosis.

6. An Integrated Approach to Identify Genetic and Environmental Mediators of Insulin Resistance.

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Insulin resistance (IR) is a common and increasing public health concern that precedes development of type 2 diabetes and cardiovascular disease. While GWAS studies have identified several loci associated with IR related traits, environmental risk factors, causal genes, and their mechanisms remain largely unknown. We therefore developed a combined experimental and computational framework to provide mechanistic insights into GWAS loci.

We first model environmental effects on gene expression in liver, fat, and skeletal muscle, three key IR tissues. Specifically, we performed RNA-seq in HepG2 (human hepatocytes), SGBS (human adipocytes), and HMCL-7304 (human skeletal myocytes) cells treated with 21 different environmental perturbations related to IR (234 total samples). We identified thousands of treatment-specific, treatment-by-cell-type-specific, and shared differentially expressed (DE) genes underlying IR response pathways. The largest number of DE genes were observed for glucose (2,853), IGF-1 (1,676), TGFa1 (1,644), and insulin (1,589) in HepG2 and for dexamethasone (3,706), IL-6 (3,129), TNF-a (2,232), and rosiglitazone (2,096) in SGBS cells. While some of the DE patterns and correlations are conserved across cell type, e.g. insulin and IGF-1 or IL- 6, TNF-a and dexamethasone, the relationship of other perturbations is dependent on the cellular context. In addition, the overall strength of the correlation seems particularly sensitive to the cell type analyzed.

We then combine these results with GWAS of IR-related traits. We show that genes differentially expressed in multiple

treatments and cells were depleted from GWAS results (p = 1.41e-02). To distinguish treatments whose association with disease are modifying risk, we compute transcriptional risk scores for each treatment in each cell-type by integrating our DE-by-treatment effects with TWAS effects generated from IR-related GWAS and GTEx eQTLs in IR-relevant tissues. We find several treatments that modify risk of IR-related traits when analyzing the contribution of environmental perturbations to insulin sensitivity and IR-related trait heritability. We further performed co-localization of IR-related trait GWAS and eQTLs from IR-related tissues to identify signals pointing to causal genes in each locus. We identified a single candidate gene for 30% of loci while in 30% of loci, multiple genes showed a positive signal.

Overall, our results provide a broad resource of the dynamics of the transcriptional landscape in IR-related tissues and demonstrate the advantages of large-scale characterization of effects of genetic variation in diversely-stimulated and pathologically-relevant cells.

7. Adiponectin Receptor 3 is Associated With Endothelial Nitric Oxide Synthase Dysfunction and Predicts Insulin Resistance in South Asians

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Type 2 diabetes (T2DM) is a global epidemic affecting over 400 million people and is a leading cause of morbidity and mortality. T2DM has a strong association with cardiovascular disease (CVD), the number one cause of death globally with 17.5 million deaths per year. A precursor of T2DM, insulin resistance is central to the development of T2DM and is a risk factor for CVD. Insulin resistance is difficult to diagnose and individuals are often untreated prior to the onset of T2DM or CVD. Despite extensive research, the genetic causes of insulin resistance and effects on the vascular system have not been adequately investigated. South Asians are more likely to have insulin resistance, diabetes and cardiovascular disease when compared to age matched European cohorts. The molecular mechanisms of why South Asians are predisposed to insulin resistance and consequently cardiovascular disease merit further investigation. We use induced pluripotent stem cells (iPSCs) derived endothelial cells to answer this important question. Endothelial cells line blood vessels of the cardiovascular system and patient specific samples are generated using patient-specific iPSCs. Unlike previous models, iPSC-derived endothelial cells (iPSC-EC) are unique because contain the individual's genetic information and the environmental influences retained in epigenetic marks are removed via reprogramming and differentiation. We have discovered biomarkers of insulin resistance that are unique to South Asians. Multiple linear regression modeling of clinical characteristics and gene and cellular phenotypic was used to develop a scoring system that predicts a patient's risk of developing insulin resistance and hence subsequently diabetes and cardiovascular complications. To our knowledge, this is the first iPSC-derived endothelial cell risk calculator with the potential to identify South Asian patients at risk for developing cardiovascular disease before disease onset, which would allow for the early implementation of interventions that prevent morbidity and mortality.

8. Contractile Stress-induced DNA Damage Contributes to the Pathogenesis of LMNA-related Dilated Cardiomyopathy

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'Nuclear mechanosensing' encompasses a wide range of biophysical pathways that are emerging as key processes in the regulation of cell function and fate. Many of these mechanisms involve the main structural protein of the nucleus, lamin-A/C (LMNA), which is abundant in stiff and mechanically stressed tissues such as cardiac/skeletal muscle, but is comparatively low in soft tissues such as the brain or marrow (Swift et al., Science 2013). LMNA's increase with tissue stiffness correlates strongly with levels of collagen-I fibers in the extracellular matrix (ECM), but mechanisms remain poorly understood and whether LMNA mechanosensing can impact genome organization and/or integrity is unknown. Here, we show that acute perturbations (~1 hr) to actomyosin stress or ECM elasticity cause rapid and reversible changes not only in LMNA protein abundance, but also in steady-state levels of DNA damage and cell cycle distribution. Embryonic hearts, iPSC-derived cardiomyocytes (iPSC-CMs) and various non-muscle cell types all show that high

actomyosin contractility can result in transient rupture of the nuclear envelope, causing cytoplasmic mis-localization of DNA repair factors and excess accumulation of DNA damage. Binucleation and micronuclei increase as telomeres shorten as a result, indicative of permanent cell-cycle arrest. Patient iPSC-CMs carrying a dilated cardiomyopathy-causing LMNA mutation likewise exhibit blebbed nuclei with elevated levels of basal DNA damage, consistent with mechanically induced nuclear envelope rupture. Deficiencies in LMNA and repair factors exacerbate these effects, but LMNA-associated defects are rescued by repair factor overexpression and also by contractility modulators currently in clinical trials (e.g., mavacamten). Contractile CMs on stiff ECM normally exhibit low phosphorylation and slow degradation of LMNA by matrixmetalloprotease-2 (MMP2), and inhibition of this LMNA turnover are seen to minimize DNA damage. LMNA is thus stress-stabilized to mechano-protect the genome.

9. Modulation of Energy Metabolism by Metformin Prevents Diet Induced Cardiac Dysfunction in a Mouse Model of Adult Congenital Heart Disease

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Objective: Congenital heart disease (CHD) is the most frequent birth defect worldwide. Improved surgical and treatment interventions have led to a significant increase in the number of adult patients with CHD, now referred to as ACHD. However the mechanisms whereby ACHD predisposes patients to heart dysfunction are still unclear. ACHD is strongly associated with metabolic syndrome, but how ACHD interacts with poor modern lifestyle choices and other comorbidities, such as hypertension, obesity, and diabetes, is mostly unknown.

Methods: We used a newly characterized mouse genetic model of ACHD to investigate the consequences and the mechanisms associated with combined obesity and ACHD predisposition and possible metabolic intervention studies by metformin.

Results: ACHD mice placed under metabolic stress (high fat diet) displayed decreased left ventricular ejection fraction. Comprehensive physiological, biochemical, and molecular analysis showed that ACHD hearts exhibited early changes in energy metabolism with increased glucose dependence as main cardiac energy source. These changes preceded cardiac dysfunction mediated by exposure to high fat diet and were associated with increased disease severity. Restoration of metabolic balance by metformin administration leads to improved liver function in both control and ACHD mice and prevents the development of heart dysfunction in ACHD predisposed mice. Metabolomic analysis of these animals revealed that metformin leads to an ACHD specific increase in metabolites associated with fat acid oxidation, likely reflecting upregulation of FAO.

Conclusions: This study reveals that early metabolic impairment reinforces heart dysfunction in ACHD predisposed individuals and diet or pharmacological interventions can be used to modulate heart function and attenuate heart failure. Our study suggests that interactions between genetic and metabolic disturbances ultimately lead to the clinical presentation of heart failure in patients with ACHD. Our current hypothesis is that metformin treatment leads to normalization of energy use by ACHD heart by enhancing FAO. This data indicates that early manipulation of energy metabolism may be an important avenue for intervention in ACHD patients to prevent or delay onset of heart failure and secondary comorbidities.

10. A Novel Patient- Specific Epicardial Device for Atrial Fibrillation Mapping

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Background: Atrial fibrillation (AF) is the most common heart rhythm disorder and impacts over 33 million patients globally. The definitive therapy for these patients is cardiac ablation to correct the underlying rhythm disorder. Despite the success of ablation, it is estimated that as many as 50% of patients will experience a recurrence of AF. The lack of success in these patients necessitates the development of new patient-specific approaches to improve treatment. Our goal is to leverage advances in 3D printing to develop patient-specific surgical devices which can precisely treat patients with AF.

Methods: A high-resolution sensor array was constructed using a patient-specific 3D printed substrate of the atrial epicardium. The sensor array deploys 256 unique electrodes across the entire atrial surface of the heart. The devices were tested on 5 different porcine animal models of AF to evaluate electroanatomic mapping capabilities on the epicardial surface of the atria.

Results: A total of 5 different devices were manufactured and tested in an iterative design process within 72 hours with a cost of $120 per device. The device demonstrated exceptional signal-to-noise ratio with the ability to discern ectopic AF foci against sinus rhythm.

Conclusions: We demonstrate the successful translation of a patient-specific 3D printed medical device into the surgical suite for the treatment of AF. This treatment modality has the potential to guide more precise treatment and reduce the rate of AF recurrence. Ultimately, these surgical tools may be translated into human clinical studies to help improve the treatment of AF

11. Understanding the Role of T Cells in the Pathophysiology of Atherosclerosis

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Background: Atherosclerosis-related diseases are a leading cause of death worldwide. Patients with atherosclerosis are at risk of developing heart attack and stroke, which may result in death. While previous murine studies have revealed how immune response regulation in T cells may play a crucial role in the promoting development of atherosclerosis, studies in humans are limited.

Objective: To genotype the CDR3 region of the T cell receptor (TCR), which is the primary binding site for the MHC-antigen complex, in T cells isolated from coronary atherosclerotic plaque.

Methods: Plaques were digested in single cell suspension and sorted into CD4+ and CD8+ T-cells in 96-well plates using fluorescence-activated cell sorting (FACS). From there, RT-PCR was performed on the RNA from the cells and two sets of nested PCR were performed specific for the α or β regions of the TCR. The DNA was barcoded then sequenced using Illumina MiSeq. This data was then used for clonality and CDR3 motif analysis.

Findings: FACS data analysis revealed significant presence of αβ T cells in the plaque of our cohort. T cells were clonally expanded in plaque, suggesting an antigen specific response. GLIPH analysis of the sequencing data revealed that patients had T cells with a shared TCR β CDR3 motif, suggesting a common antigen.

Conclusion: Our data suggests there is an antigen specific response by T cells in coronary atherosclerotic plaque. Further investigation is required to identify potential disease-causing antigens that activate these T cells.

12. Cost-effectiveness Analysis of Fenestrated Endovascular Aortic Repair Compared to Open Surgical Repair for Juxtarenal Abdominal Aortic Aneurysms

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Over the past decade, fenestrated endovascular aortic aneurysm repair (fEVAR) has emerged as an alternative to open surgical repair (OSR) to treat juxtarenal abdominal aortic aneurysms (jAAA). The clinical effectiveness of the strategy has been well described, but there is a knowledge gap regarding where this new endograft technology lands on the cost-effectiveness frontier in the American market.

A decision analytic and Markov model was constructed to evaluate the costs and quality adjusted life years (QALYs) from a healthcare perspective and calculate the net monetary benefit (NMB; $/QALY) and incremental cost-effectiveness ratio (ICER; difference in costs divided by difference in QALYs) comparing the two interventions.

Costs were derived from the 2017 Medicare reimbursement schedule and utilities extracted from secondary data. Clinical probabilities of short- and long-term mortality, perioperative complications, and early and late interventions were obtained through literature search and input as weighted-averages. A base case CE analysis of a 65-year-old male and one- and two-way sensitivity analyses were conducted.

13. Engineering a Rodent TRPV1 Receptor with Qualities of the Chicken Modulate Calcium Influx and Mitochondrial Function

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Birds, unlike mammals, are resistant to the hot sensation when consuming chili peppers, which allows birds to disperse these seeds from a pepper in nature. The inability for birds to sense the hot sensation is accredited to genetic divergence in the transient receptor potential vanilloid 1 (TRPV1), also known as capsaicin receptor, compared to mammals[1] .

TRPV1 is a nociceptive, non-selective cation channel, with a high permeability for Ca2+, that activated by capsaicin, heat, protons and other endogenous lipids[2]. Besides traditional pain sensation, TRPV1 can act as a general cellular stress sensor [3]. Here, we tested our hypothesis that a K710N point mutation in rodent TRPV1, which mimics the chicken TRPV1 sequence, is a crucial mediator of calcium influx and mitochondria function under cell stress.

14. The Effect of Digital Physical Activity Interventions on Daily Step Count: A Randomised Controlled Crossover Substudy of the MyHeart Counts Cardiovascular Health Study

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Smartphone app-delivered interventions could increase physical activity, but few randomised trials have tested this hypothesis. The MyHeart Counts Cardiovascular Health Study is a longitudinal smartphone-based study with the aim of elucidating the determinants of cardiovascular health. We aimed to investigate the effect of four different physical activity coaching interventions on daily step count in a substudy of the MyHeart Counts Study. We recruited adults (aged ≥18 years) in the USA with access to an iPhone smartphone who had downloaded the MyHeart Counts app. After completion of a 1 week baseline period of interaction with the MyHeart Counts app, participants were randomly assigned to receive one of 24 permutations (four combinations of four 7 day interventions) in a crossover design. Interventions consisted of either daily prompts to complete 10,000 steps, hourly prompts to stand following 1 h of sitting, instructions to read the guidelines from the American Heart Association website, or e-coaching based upon the individual's personal activity patterns from the baseline week. The primary outcome was change in mean daily step count from baseline, assessed in the modified intention-to-treat analysis set, which included all participants who had completed 7 days of baseline monitoring and at least 1 day of one of the four interventions. This trial is registered with ClinicalTrials.gov, NCT03090321. Between Dec 12, 2016, and June 6, 2018, 2783 participants consented to enrol in the coaching study, of whom 1075 completed the criteria for the analysis set. All four interventions significantly increased mean daily step count from baseline (mean daily step count 2914 [SE 74]). These findings suggest that digital interventions delivered via an app have the ability to increase short-term physical activity levels in a free-living cohort.

15. Exosomal miR-106a-363 Cluster Enhances Endogenous Myocardial Repair by Stimulating Cardiomyocyte Cell Cycle Re-entry in the Ischemic Myocardium

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BACKGROUND: It is well-known that mammalian cardiomyocytes rapidly proliferate during the embryonic development but exit the cell cycle soon after birth. This represents one of the major limitations in attenuating myocardial injury. There have been significant efforts to study the cell cycle re-entry and adult cardiomyocyte proliferation; however, the exact

relationship between restoration and cell cycle re-entry of the injured cardiomyocytes is poorly understood.

HYPOTHESIS: The exosomal miR-106a-363 cluster (miR cluster) restores the heart from ischemic damage by stimulating cardiomyocyte cell cycle re-entry.

METHODS AND RESULTS: In our previous study, we found exosomal miR 106a-363 cluster secreted from hypoxia injured iPSC-derived cardiomyocytes (iCMs) restored the ischemia injured iCMs in vitro and murine myocardium in vivo. To verify the mechanism of miR cluster, we analysed the mRNA expression regulated by miR cluster using RNA-Seq. We identified that the Notch3 is a direct target gene of miR cluster by luciferase reporter assay. RNA-Seq analysis revealed that this cluster regulates the genes involved in cell contraction, DNA replication and cell cycle G1-S transition. qRT-PCR analysis confirmed significantly increased expression of positive cell cycle regulators of G1/S transition (Cyclin D, CDK4) and G2/Mitosis phase (PLK-1, Cyclin A) and significantly decreased expression of negative regulators, including p21cip1 and p27kip1 (CDKIs) in in the miR cluster and/or siNotch3 transfected iCMs when compared to the control groups. Furthermore, we observed significantly increased expression of other regulators of cell cycle: EdU incorporation (S phase), Ki-67 (G1, S, G2 and M phase), and Aurora B (G2 and M phase) when compared to the control groups (fold change; 3.468±0.343, 6.583±2.156, and 3.084±1.702. respectively, $p<0.0001$).

CONCLUSION: Our data from the hypoxia-injured iCMs suggest that there is persistent exosomal expression of the miR cluster to stimulate the cardiomyocytes to re-enter cell cycle via Notch3 signaling pathway to repair ischemic injury. This miR cluster simulates endogenous repair to enable rapid translation of precision medicine in heart failure patients.

16. Design-based Model of the Mitral and Aortic Valves and Simulations of Patient-specific Left-ventricular Flow

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This work presents a method for modeling patient-specific left-ventricular blood flow and mitral valve dynamics. We aim to build a model that produces realistic flows under physiological pressures and can be constructed without excised specimens. Patient-specific left-ventricular geometry and deformation through the cardiac cycle are measured from MRI scans. We construct model mitral and aortic valves using a design-based methodology, in which we compute the loads the valve must support, then assign model geometry and material properties accordingly. The system is then simulated with blood using the immersed boundary method. With this model, we aim to study macro-scale cardiac flows and pathophysiology.

17. Identification of Novel Genetic Mechanisms Involved in Development and Progression of Non-alcoholic Fatty Liver Disease

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Non-alcoholic fatty liver disease (NAFLD) is the most prevalent form of chronic liver disease, characterized by excess fat accumulation in the liver without a history of excessive alcohol consumption. Patients with NAFLD often progress to an advanced stage of non-alcoholic steatohepatitis (NASH) and ultimately irreversible end-stage liver disease. To date, there are no available pharmacological treatments against NAFLD and NASH.

The overall aim of this study is to investigate genetic mechanisms regulating lipid accumulation and lipotoxicity in hepatocytes, which will help us understand the cause of NAFLD and NASH. We treated human hepatocytes (HepG2) with the two most abundant dietary plasma fatty acids; a saturated fatty acid, palmitic acid (PA), and a monounsaturated fatty acid, oleic acid (OA); and TNF to study their impact on steatosis, apoptosis and inflammation, as well as gene expression, with the goal of developing an in vitro model for NAFLD development and progression.

First, we showed that OA markedly induced lipid accumulation, as well as mRNA expression of the lipid droplet binding protein, Plin2. In contrast, PA had little effect on lipid accumulation, but significantly activated the Caspase 3/7 apoptotic and NF-kB p65 inflammatory pathways. Second, we performed RNA-seq and analyzed differential expression (DE) using edgeR. We identified 91, 945, and 1,795 differentially expressed genes after OA, and PA and TNF treatment, respectively,

at false discover rate (FDR) <0.01. We further performed gene-ontology (GO) and gene-disease association (DisGeNET) analyses using the Bioconductor clusterProfiler and enrichplot packages. In addition to genes from lipid metabolism pathways, genes enriched for disease categories related to the fatty liver-associated gene signature of HepG2 cells and the activation of the response to lipid accumulation and lipotoxicity were upregulated with OA, PA and TNF treatments.

Our preliminary results indicate that perturbations with OA, PA and TNF induce differences in cellular phenotypes and gene expression patterns that closely resembles development and progression of NAFLD and NASH. Next, we plan to perform further molecular profiling using chromatin accessibility and interaction methods in our system and to integrate findings from human genetics to advance knowledge about NAFLD and NASH.

18. Multi-Omics Investigation of Cardiomyocyte-to-Fibroblast Crosstalk In Human iPSC Models

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Introduction: Cardiac cells communicate with each other in part through secreted proteins known as cardiokines. The total repertoire of proteins secreted by cardiac cells is unknown. We aim to apply human iPSC models and proteomics to identify secreted proteins and the crosstalk signals they mediate.

Method: We optimized an experimental protocol to recover and identify cell-specific secreted proteins from human iPSC-cardiac cells. Human iPSCs were differentiated into cardiomyocyte (CM) and endothelial cells (EC) using established protocols, after which secreted proteins were extracted from the conditioned medium for analysis. We combined multiplexed aptamer-based proteomics and high-resolution mass spectrometry to identify secreted proteins from iPSC-CM, iPSC-ECs, and primary ventricular fibroblasts. An in-silico filter was implemented to prioritize bona fide cardiokines over proteins externalized by passive lysis.

Result: We identified 146 candidate cardiokines at 1% false discovery rate, including cell-specific cardiokines as well as a common core secretome of three cardiac cells. We analyzed the data to identify potential signals secreted by cardiomyocytes to mediate fibroblast function, using a ligand-receptor model based on transcriptomics and proteomics data to prioritize cardiokines secreted by iPSC-CMs and which bind to fibroblast-expressed receptors. To examine their roles in fibrosis regulation, we selected three candidate cardiokines (PLAU, FGF7, and CXCL12) for verification using immunodetection, and exposed their recombinant proteins at multiple concentrations to human ventricular fibroblasts. The results nominated cardiokine-specific effects on recipient cell gene expression including evidence of modulation of fibroblast transcription and translation pathways.

Conclusion: We demonstrate a proteomics approach in iPSC models to explore the large-scale human secretome of multiple cardiac cell types. The approach holds promise for identifying disease markers and understanding intercellular communication in cardiac development and diseases.

19. Glycogen Synthase Kinase 3b Inhibition Enables Massive Expansion of hiPSC-derived Cardiomyocytes in a Cell Density-dependent and Nuclear Yap-independent Manner

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Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) serve as an invaluable tool for cardiac disease modeling and patient-specific drug screening. However, the limited proliferative capacity of fully differentiated hiPSC-CMs delays translation of such technology to industry where the massive number of cardiomyocytes are needed. While previous studies have shown glycogen synthase kinase 3b (GSK3b) inhibitor, CHIR99021-mediated Wnt activation leads to cardiomyocyte proliferation, the extent of proliferation has been reported to be limited (~4-fold). We demonstrate hiPSC-CMs can be massively expanded up to 200-fold by GSK3b inhibition and continuous passaging. Reducing direct cell-cell contact is essential for enabling continuous hiPSC-CM proliferation. Furthermore, contact-mediated inhibition of hiPSC-CM proliferation is uncoupled from Hippo-YAP activity. We envision this simple yet robust method can facilitate the translation of hiPSC-CM technology from research to market.

20. ALDH1A3 Inhibition Protects Against Hyperproliferation of Pulmonary Arterial Smooth Muscle in Pulmonary Arterial Hypertension

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Understanding the mechanisms of hyperproliferation in pulmonary arterial smooth muscle cells (PASMC) that occlude the lumen of pulmonary arteries, could lead to therapies for pulmonary arterial hypertension (PAH). The hyperproliferative phenotype is in part result from altered cell metabolism and gene modification.

From transcriptomic analysis in nine donor control and 12 PAH patient, the heightened ALDH1A3 is found in PAH patients. The increased level is verified at mRNA and protein level in PAH vs. control PASMC. The heightened ALDH1A3 at protein level is observed in control PASMC under hypoxia as well as in PASMC of mice with pulmonary hypertension (PH) hypoxia. PH is retarded by deleting Aldh1a3 in SMC in these mice. Decreasing ALDH1A3 by using siRNA reduces the hyperproliferation and glycolysis of PAH PASMC. The declined downstream metabolite acetyl-CoA is detected in the nuclei of PAH PASMC with siALDH1A3. siALDH1A3 also reduced the elevated H3K27ac in PAH PASMC. Transcriptomic analysis in PAH PASMC with siControl vs siALDH1A3 demonstrates the reduced cell cycle and metabolic genes with siALDH1A3. These genes have NFY binding site from motif enrichment analysis. From H3K27ac ChIP-seq analysis in the same samples, we found the decreased density of H3K27ac in the site of NFY, cell cycle genes CCNB2 and TTK, as well as metabolic genes PKM2 and DLD.

We showed that ALDH1A3 enhanced the proliferation associated metabolic gene expression linked to histone acetylation. This study reveal a novel mechanism for understanding the biological process and may establish a potential promising target for PAH therapy.

21. Characterization of Heterogeneity in Ddipogenesis by Single-cell RNA-seq

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Significant cell-to-cell variability in morphology and function has long been observed in differentiating adipocytes, which cannot be discerned by population-averaged measurements, such as bulk RNA-seq. It is also unclear to what extent the observed variability reflects the presence of phenotypically distinct subpopulations or asynchronous cellular responses to differentiation stimuli. To better characterize the heterogeneity in adipogenesis, we performed single-cell RNA-seq on human Simpson–Golabi–Behmel syndrome (SGBS) cells - a non-immortalized and non-transformed preadipocyte model - before and after 7 days of adipogenic differentiation. By using droplet-based single cell capture which enabled the gentle handling of large and rupture-prone adipocytes, we successfully mapped a single-cell profile including 5,662 cells from day 0 and 3,643 cells from day 7, validated by the correlated transcriptomic profiles of matching bulk samples. Next, by using K-means clustering on the aggregated and normalized single-cell data of both timepoints, followed by t-SNE visualization, we defined 3 major clusters with distinctive gene expression patterns. Cells in Cluster 1 showed strong enrichment in genes featuring a preadipocyte phenotype, including those involved in proliferation (TGFB1, CCND1), extracellular matrix remodeling (CLDN11, SERPINE1) and negative regulation of adipogenesis (GREM1, ID3). Cells in Cluster 2 and 3 were enriched in genes positively associated with adipogenesis (FABP4, ADH1B, CFD), indicating a cell commitment to adipogenic trajectory. Among these, cells in Cluster 3 were further enriched in genes characteristic of a mature adipocyte function, such as adipokine synthesis (ADIPOQ), lipolysis (PLIN1, LIPE, G0S2, PNPLA2), fatty acid biosynthesis and desaturation (FASN, SCD, FADS1), representing a subpopulation of lipid-rich and metabolically active adipocytes further along into differentiation. Our single-cell transcriptomic catalog thus serves as a valuable resource to uncover novel molecular events underlying the intrapopulation heterogeneity both within and between timepoints of adipogenesis. Future studies combining single-cell profiling with genetic and metabolic perturbations in human primary (pre)adipocytes can pave a way for deeper understanding of subject-, depot-, and cell-specific regulation of adipocyte formation and function in health and disease.

22. Effects of Cardiomyopathy Mutations and Small Molecule Drugs on the Load-dependent Kinetics of Single Molecules of Human b-cardiac Myosin

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Cardiomyopathy-causing mutations and small molecule drugs can alter contractility of the heart muscle. Here, we investigate the underlying mechanism at the level of individual molecules of human b-cardiac myosin, the isoform responsible for power production in the ventricles of the heart. A key parameter of contractility is the detachment rate of myosin from actin. This rate determines the time that myosin is bound to actin in a force-producing state and, importantly, depends on the load (force) against which myosin works. Here, we measure the detachment rate of single molecules of human b-cardiac short-subfragment-1 (sS1) and its load-dependence. We find that both can be modulated to various extents by both small molecule compounds and cardiomyopathy-causing mutations, and effects of mutations can be reversed by introducing appropriate compounds. Furthermore, single-molecule dosage analysis of omecamtiv mecarbil (OM), a drug in phase-III clinical trials for treatment of heart failure, reveals its key mechanism to activate the heart. Given our measurements of the detachment kinetics, we calculate the resulting duty ratio, average force, and average power of single myosin molecules, and we find a striking separation between activating vs. inhibitory perturbations consistent with physiological expectations of hyper- and hypo-contractility. Our results suggest that cardiac contractility can be controlled by tuning the load-dependent kinetics of single myosin molecules.

23. Identifying the Molecular Mechanisms of Physical Activity on Vascular Function in Type 2 Diabetes

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Type 2 diabetes mellitus (T2DM) is a condition in which the body fails to either produce enough insulin or use its insulin properly, preventing glucose uptake and storage in cells. T2DM is a growing epidemic with increasing prevalence and incidence in the U.S. due to a rise in sedentary lifestyle and the regular consumption of excess added sugars. Hyperglycemic stress has been proven to contribute to endothelial cell (EC) dependent vascular dysfunction, which represents the main precursor to cardiovascular disease (CVD), the leading cause of mortality among patients with cardiometabolic disease. Structured exercise programs are known to improve glucose control in T2DM patients, however ~15-20% of T2DM patients fail to exhibit glucose improvements with exercise. Studies demonstrate that both genetic (single nucleotide polymorphisms) and epigenetic (DNA methylation) mechanisms contribute to the response to exercise training. Our goal is to identify and understand the precise molecular mechanisms contributing to cardiovascular health by employing induced pluripotent stem cell (iPSC) technology, and patient-specific endothelial cells (iPSC-ECs) derived from these iPSCs. We hypothesize that T2DM exercise non-responders exhibit EC dysfunction when subjected to hyperglycemic challenge. For this, we have analyzed the functional and transcriptomic profiles of iPSC- ECs. Our findings will have a broad scientific impact enabling a better understanding of diabetic cardiomyopathy, and the specific beneficial effects of exercise on the diabetic endothelium. The proposed study will have significant clinical impact by enabling targeted utilization of exercise as a therapeutic tool in T2DM patients.

24. Single-Cell RNA-Seq and Patient-Specific iPSCs Reveal Endocardial Abnormalities in Hypoplastic Left Heart Syndrome

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Hypoplastic left heart syndrome (HLHS) is one of the most devastating forms of congenital heart defects. Previous studies have only focused on intrinsic defects in the myocardium. However, this does not sufficiently explain the abnormal development of cardiac valve, septum, and vasculature, which are known to originate from the endocardium. Here, using single-cell RNA profiling, induced pluripotent stem cells, and fetal heart tissue with underdeveloped left ventricle, we identified a developmentally impaired endocardial population in HLHS. The intrinsic endocardial deficits contributed to abnormal endothelial to mesenchymal transition, NOTCH signaling, and extracellular matrix organization, all of which are key factors in valve formation. Consequentially, endocardial abnormalities conferred reduced proliferation and maturation of cardiomyocytes through a disrupted fibronectin-integrin interaction. Several known HLHS de novo mutations all contributed to the abnormal endocardial gene expression through the alteration of promoter activities. These mechanistic discoveries provide an alternative angle for early intervention and heart regeneration in HLHS.

25. E-Cigarette Vapor Accelerates Abdominal Aortic Aneurysm in Mice

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Significance: Abdominal aortic aneurysm (AAA) disease is common, morbid and highly lethal with strong tie to tobacco use. To date, the molecular mechanisms underlying the relationship between smoking and AAA disease have been minimally explored. Importantly, there is currently no information regarding the effects of electronic (e-)cigarettes. E-cigarettes and vaping have gained popularity with all ages, sexes and races, as potentially "healthier" substitutes for smoking while still delivering nicotine at sufficient levels to satisfy addiction. Other major components include propylene glycol and glycerine (liquid e-juice), and added flavorings. This work explores the effects of e-cigarette vapor with and without nicotine on experimental abdominal aortic aneurysm.

Methods: 10-week-old mice of both genders were exposed to daily e-juice (50/50%) vapor with or without nicotine (24 mg/ml), 9 puffs/min for 1 hour/day for 6 weeks. After 2 weeks of exposure, mice received a local infusion of porcine pancreatic elastase into the infrarenal region of the aorta (PPE infusion model). During 28 days of follow up, aneurysm growth was tracked via ultrasound and blood pressure measurements via tail-cuff method were obtained. Harvested aneurysmal tissues were examined for analysis of known key AAA genes.

Results: We found that exposure to e-juice vapor containing nicotine augments experimental AAA formation compared to e-juice vapor exposure alone. When compared to room air-exposure, e-juice vapor alone also led to enhanced aneurysmal growth. This was consistent for both genders, with more pronounced effects in males. There were no changes in blood pressure between treatment groups. Key genes involved in aneurysm-related vascular inflammation and remodeling, including matrix metalloproteinases (MMPs), interleukin-6 (IL-6) and C-C motif chemokine ligand 2 (CCL-2) were upregulated in aortic tissue in response to e-cigarette vapor containing nicotine (vs. juice or room air).

Conclusion: Inhaled e-cigarette vapor augments PPE model AAA, accompanied by enhanced regulation of genes known to be involved in AAA development. While adding nicotine leads to more pronounced effects, e-juice vapor alone can lead to enhanced aneurysmal growth compared to room air.

26. Beat-to-beat Computational Assessment of Cardiac Function Using Labeled Echocardiogram Videos and Weakly Supervised Deep Learning Segmentation

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Accurate assessment of cardiac function is crucial for the diagnosis of cardiovascular disease and deciding clinical management, however human assessment of cardiac function has high variance and can miss subtle cardiac dysfunction. To overcome this problem, we present a video-based deep learning system trained on echocardiogram videos labeled with human calculations of ejection fraction and weakly supervised segmentation of the left ventricle. Our models segmented the left ventricle with near human accuracy (Dice similarity coefficient of 0.84) and predicted ejection fraction at expert human accuracy (mean absolute error of 5.4%, RMSE 6.16, R2 = 0.71). Prediction of heart failure with reduced ejection fraction from video alone was highly accurate (AUC of 0.94). In the cohort of patients with predicted reduced ejection fraction but discordance with human assessment, a clinical reduction of ejection fraction was seen in patients on subsequent study. Our results show that our machine learning system reproduces human assessment of cardiac function and accurately predicts subclinical change in ejection fraction, laying the foundation for more precise assessment of cardiac function.

27. Single-cell RNA-seq Unveils Unique Transcriptomic Signatures of Organ-Specific Endothelial Cells

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Endothelial cells (ECs) display considerable functional heterogeneity depending on the vessel and tissue in which they are located. While these functional differences are presumably imprinted in the transcriptome, the pathways and networks which sustain EC heterogeneity have not been fully delineated.

To investigate the transcriptional control of EC specification, we analyzed single-cell RNA-sequencing (scRNA-Seq) data from tissue-specific mouse ECs generated by the Tabula Muris consortium. We found a strong correlation between tissue-specific EC transcriptomic measurements generated by either scRNA-Seq or bulk RNA-Seq, thus validating the approach. Using a graph-based clustering algorithm, we found that certain tissue-specific ECs cluster strongly by tissue (e.g. liver, brain) whereas others (i.e. adipose, heart) have considerable transcriptional overlap with ECs from other tissue. Using gene set enrichment analysis, we identified novel markers of tissue-specific ECs and signaling pathways that may be involved in maintaining their identity. By performing pseudotime trajectory analysis, we found that ECs from endoderm-derived tissues appear to be more developmentally immature when compared with the highly specialized ECs of ectoderm-derived tissues such as brain. In addition, we compared these data from mouse with human fetal heart scRNA-seq data for interspecies correlation in organ-specific EC gene expression. Finally, we identified potential angiocrine interactions between tissue-specific ECs and other cell types by analyzing ligand and receptor expression patterns.

In summary, we have utilized scRNA-Seq to uncover transcriptional networks which maintain EC identity and identify novel developmental and angiocrine relationships between tissue-specific ECs.

28. A Multidimensional Gender Based Study on UCSF Electronic Medical Records to Improve Women 's Health in Cardiovascular Disease

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Problem: Multi-vessel and left main coronary artery disease (CAD) is the leading cause of death among women in the United States. CVD is the cause of ischemic heart disease (IHD), Reduction of blood flow to the heart muscle due to a build-up of plaque in the coronary arteries of the heart. It is most common in cardiovascular diseases.

According to the American Heart Association's released 2018 update on heart disease and stroke statistics, CVD claimed 399,028 female lives in 2017, which is more lives than those lost to all types of cancer and chronic lung disease combined.

Data from the National Health and Nutrition Examination Survey (NHANES) indicates that approximately half of the 27.6 million American adults living with CVD are women. The majority (more than 8 million) of these women have ischemic heart disease (IHD), which includes both obstructive and nonobstructive coronary artery disease and acute coronary syndromes. In addition, a significant number of women are living with heart failure (> 3 million), pulmonary hypertension (> 3 million), and congenital heart disease (0.3-0.6 million).

Hypothesis: They are several reasons behind the bad outcome in CVD in women versus men. Women are not treated at the right time and right way right for CVD. Personalized medicine for have a better guideline is a solution. There are several bottlenecks from the time of Diagnostic to Treatment based on Gender.

29. Substrate Elasticity Impacts Duchenne Muscular Dystrophy Cardiomyopathy Progression

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Duchenne Muscular Dystrophy (DMD) is a X-link disease affecting ~1:3500 boys per year and culminating with heart failure in early adulthood. DMD results from >200 possible genetic mutations on dystrophin. The lack of dystrophin disrupts the anchoring of the cell sarcomere to the extracellular matrix (ECM), affecting the cardiac contraction. With

disease progression, in an attempt to mitigate the subcellular defects, the tissue stiffness and ECM composition remodel in association with a dilated cardiomyopathy phenotype and fibrosis. Our hypothesis is that disease progression is accelerating because of this remodelling, through a positive feedback loop involving multiple mechanosensing pathways. Here, we use a single-cell assay platform to model the effect of fibrotic remodelling in DMD. This platform allows measuring the force production of single human-derived pluripotent stem cell (hiPSC) cardiomyocytes (CMs). The substrate stiffness can be controlled to match that of healthy (~10kPa) or fibrotic (~35kPa) tissue. In addition, single iPSC-CMs are patterned in an elongated 1:7 aspect ratio using microcontact printing of ECM protein. This enhances their intracellular structural maturity towards a more mature adult phenotype. This renders our in-vitro model more representative of the human pathology and greatly improves our measurements standardization. Our results show that single iPSC-CMs with DMD mutations have a dramatically reduced ability to produce force on stiffer substrates compared to their isogenic control. This loss of contractile function correlates with an increase in reactive oxygen species (ROS) and mitochondria dysfunction, as well as with other markers of stress response and cellular senescence. We are asking how the remodeling of the ECM stiffness and composition is signaled from the outside-in and affects the progression of the disease phenotype. The further development of our platform and approach will allow for more accurate in-vitro modeling of cardiac diseases and greatly increase our understanding of the underlying biophysics of mechanosensing.

30. Salt Intake and Cardiovascular Outcomes in African Americans With Hypertensive Chronic Kidney Disease

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Excessive sodium intake is associated with higher blood pressure (BP).However, some studies show higher risks of death and cardiovascular events at lower levels of sodium intake. Limitations of many of these studies include use of a single 24 hour urine collection to estimate sodium intake, or imbalances in age, race or other important clinical factors across categories of sodium intake. In contrast, the African American Study of Kidney Disease and Hypertension (AASK) was comprised of self-identified African Americans with CKD and it collected multiple 24-hour urine sodium samples. We used data from AASK to evaluate the association of sodium intake with BP, left ventricular mass, and cardiovascular events among African Americans with hypertensive CKD. We used linear regression to examine the association of sodium excretion with BP and left ventricular mass and Cox proportional hazards regression models to evaluate the association of sodium excretion with the cardiovascular composite endpoint. Of the 691 participants from the AASK trial phase who enrolled in the cohort phase, 647 had ambulatory blood pressure measurements available (mean number of 24-hour urine collections = 9.8, +/-2.6). During a mean follow-up time of 7.1 (SD 3.4) years, there were 354 cardiovascular endpoints. We found no significant association of sodium excretion with the cardiovascular composite endpoint. Each 1-gram increase in 24-hour sodium excretion was associated with 1.3 / 0.9 mm Hg increase in systolic / diastolic BP. Higher urinary sodium excretion was associated with higher left ventricular mass in 2D imaging in adjusted models.

31. Cushioned–Density Gradient Ultracentrifugation (C-DGUC) Improves the Isolation Efficiency of Exosomes for their use in Cardiovascular Disease Research

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Background and Purpose: Ultracentrifugation (UC) is recognized as a robust approach for the isolation of exosomes especially when combined with a second step that involves density gradient ultracentrifugation (DGUC). However, recent studies have highlighted limitations associated with the use of UC including low recovery efficiencies and possible aggregation of exosomes. Such effects could subsequently impact the use and study of exosomes in biological systems and as disease biomarkers.

Methods: We tested the benefit of using a liquid cushion of iodixanol during the first UC step to improve the yield of exosomes that are concentrated from the conditioned media (CM) of J774.1 murine macrophages in a method we recently termed Cushioned(C)-DGUC. We also compared the yield and purity of exosomes isolated by C-DGUC with those isolated by first subjecting CM to two other forms of concentration that included: ultrafiltration (UF) and polyethylene glycol (PEG) sedimentation prior to DGUC. Lastly, we applied the use of C-DGUC for the isolation of EVs from human plasma for biomarker studies of peripheral arterial and aneurysmal diseases at UCSF.

Results: Our data show that the concentration step largely determines the yield and purity of exosomes isolated following the second DGUC step. The use of a high-density iodixanol cushion in cushioned-UC (C-UC) led to a threefold improvement in exosome yield over conventional UC. Although subjecting the CM to UF resulted in a similar exosome recovery efficiency, it retained eight-fold more soluble proteins than C-UC method. Strikingly, PEG precipitation of the CM generated a substantial number of non-exosomal nanoparticles, which could not be efficiently eliminated by the DGUC step. Western blot analysis reproducibly detected exosome markers CD-81, TSG101 and Alix in fractions 6 and 7. Finally, using C-DGUC to fractionate human plasma led to the separation of EVs from HDL that are a confounding source of extracellular RNA.

Conclusions: Collectively, our data demonstrate that the use of a high-density liquid cushion of iodixanol during the concentration step of C-DGUC substantially improves the yield and purity of exosomes derived from cell culture media and complex biofluids including plasma. This approach has improved our ability to initiate functional studies of macrophage-derived exosomes as contributors to atherosclerosis and to its resolution. It has also led to biomarkers studies of peripheral arterial disease severity and aneurysmal disease among patient cohorts at the UCSF vascular clinics.

32. Anatomically Inspired Annuloplasty Ring for Mitral Valve Repair

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Mitral Valve (MV) Regurgitation Disruption to the MV apparatus results in insufficient leaflet coaptation, leading to MV regurgitation Backflow of blood from the left ventricle into the left atrium during contraction decreases overall pumping efficiency, resulting in fatigue and heart failure.

MV annuloplasty (Fig 2) is the most common surgical repair technique and aims to increase leaflet coaptation and restore healthy valve function (AL Gillinov et al., 1998)

Limitations with existing annuloplasty devices:

Many annuloplasty rings commercially available, varying in size, shape, and rigidity.

Many rings are rigidly fixed in a 3D conformation, thus hindering natural annulus dynamics. (E Lansac, 2002)

Elimination of annulus dynamics can lead to undesirable tissue strains that lead to suture dehiscence and repair failure over time. (MS Sacks, 2006)

Design Specifications

Hypothesis: an annuloplasty ring with similar biomechanical properties to those of a healthy annulus will appropriately restore leaflet coaptation while allowing for physiological annulus dynamics.

• Previous studies demonstrate the biomechanical importance of non-uniform material properties along the annulus (KD Lau, 2010)

• Prototype features a super-elastic nitinol core over-molded with urethane segments of varying stiffness to match annulus YM values in literature (GM Gunning, 2014) (Fig 3A,C)

• To maintain desirable superelastic material properties, NiTi is shape-set using electrical resistance heating rather than oven furnace (Fig 3B)

• First-gen device features a PTFE microfilament to simulate constrained ante-posterior distance and induce saddle shape during radial contraction

33. An Electromagnet-based Epicardial-endocardial Alignment System for Constant Force Bipolar Ablation

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Objective: This study aimed to develop an electro/magnetically enabled epicardial-endocardial ablation alignment system capable of pairing ablation elements on two sides of the myocardium. We performed evaluation of proportional-

integral-derivative feedback controllers for the purpose of maintaining constant contact force during ablation of variable thickness tissue.

Methods: We constructed a bench-top magnetic test environment for an epi-endo ablation alignment system using rare neodymium and electro-magnets. A bench-top model was designed to perform magnetic strength testing across variable thickness. Rare earth neodymium magnets were used as endocardial system complementary to a fixed or variable electromagnet epicardial system. Force measurement was performed between the coupled system while the epicardial magnet was guided using an automated control system. A proportional-integral-derivative controller was used to maintain force within a predefined range through voltage alteration of electromagnetic strength.

Results: We have engineered a varying magnetic strength mechanism capable of maintaining constant contact force that can be guided along a path of variable thickness. The PID controlled electromagnetic epicardial catheter had superior performance compared to the fixed magnetic strength system with increased percentage of time within a target force range. We demonstrate the ability to perform tissue thickness characterization via electromagnetic force to manipulate magnetic strength for maintaining constant force on the tissue during ablation.

Conclusion: We report on a proof-of-concept electromagnetic catheter ablation system with controlled feedback capable of varying magnetic field strength for the purpose of contact force ablation.

34. Continuous Non-Invasive Blood Pressure Monitoring in Neonates Using a Wearable Capacitive Sensor

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Objectives: To evaluate the feasibility of a wearable continuous non-invasive blood pressure (cNIBP) monitoring device in critically ill neonates.

Design: Prospective, observational pilot study

Setting: Neonatal intensive care unit (NICU)

Patients: Eleven critically ill but normotensive neonates with umbilical arterial catheters. Their gestational age ranged from 26-6/7 to 40-1/7 weeks and weight ranged from 0.9 kg to 3.6 kg

Interventions: Novel capacitive sensors placed on wrist and foot at radial and/or dorsal pulse points to obtain pulse waveform measurements for up to 10 hours from each infant. The measured changes in capacitance from the cNIBP sensor were wirelessly transmitted to an Android application. The Android application used proprietary and highly efficient artificial neural network (ANN) algorithms to derive SBP, DBP, and MAP values from measured pulse waveform data. These data were then compared with corresponding umbilical arterial line data to determine the accuracy of these derived BPs.

Measurements and Main results: Approximately 100 hours of cNIBP data from eleven patients was correlated with corresponding arterial line data. Bland-Altman plots for systolic (SBP), diastolic (DBP), and mean arterial blood pressure (MAP) show mean average errors (MAE) < ±5 mmHg with standard deviations (SD) <8 mmHg.

Conclusions: The cNIBP sensor can non-invasively monitor the blood pressure of neonates who are less than a week old with an accuracy within FDA specifications. Our study is limited to critically ill but normotensive neonates.

35. Prenatal Exposure of Cigarette Smoke Impacts Cardiac Regeneration

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Background: Neonatal mammalian hearts have the unique characteristic to fully repair and regenerate following injury. This discovery has led to visionary endeavors to understand and subsequently reawaken regeneration processes in the human adult heart that are lost after birth, contributing to heart failure and death after ischemic insults. In utero

exposure to tobacco smoke has detrimental effects on fetal development and growth. However, the consequences of prenatal exposure to cigarette smoke on murine neonate's cardiac regeneration have never been explored.

Methods: To study the impact of cigarette smoke during the entire pregnancy on cardiac regeneration in the offspring, plugged female wild type C57LB/6N mice were exposed either to cigarette smoke (n=6) or filtered air (control, n=6). Two days after birth, 5 pups from each litter were assigned to the two experimental groups: Myocardial infarction (MI) and sham. Detrimental effects of in utero exposure to tobacco smoke on recovery of cardiac function following MI surgery were followed using two dimensional speckle tracking echocardiography and strain imaging. To investigate the underlying mechanism, cardiac tissue of the infarct and remote zone was collected for non-coding RNA analysis.

Results: In utero exposure to cigarette smoke significantly compromised cardiac regeneration in neonates. At both early and late time points in the phase of cardiac repair, hearts of neonates exposed to cigarette smoke during pregnancy showed a marked impairment of cardiac regenerative potential. In particular, ejection fraction, fractional area change and shortening, as well as left ventricular internal diameter during systole remained pathologically changed following MI insult in the smoked group until the very endpoint.

Conclusions: Collectively, we here provide evidence that in utero exposure to cigarette smoke strongly compromises cardiac regeneration in the newborn offspring. This result reinforces smoking cessation during pregnancy. Additionally, understanding the changes in non-coding RNA expression in a setting of preserved versus disrupted repair of the heart might be an important first step towards the identification of key cellular processes in cardiac regeneration after injury.

36. Biomimetic Injectable Hydrogels with Tunable Mechanical and Biochemical Properties for Cell Transplantation in Peripheral Arterial Diseases (PAD)

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Peripheral arterial disease (PAD) affects over 8 million people in the US; it is associated with narrowing the peripheral arteries and restricts the blood flow to peripheral limbs. Current treatments of PAD such as cytokine therapy have shown limited long-term angiogenic benefits in clinical trials. Human induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) have demonstrated promise in treating PADs in preclinical models; however, low transplantation viability hampers the therapeutic potential of this approach. To address the massive cell death post-transplantation, a vehicle that protects cells during delivery and provides functional support is required. A family of genetically engineered extracellular matrix (e-ECM), termed elastin-like protein (ELP), are developed using standard recombinant protein technology. The e-ECMs are composed of altering elastin-like structural sequences (VPGXG) and customizable bioactive peptide sequences (e.g., RGD, YIGSR and DGEA). ECM scaffolds provide essential signaling cues and modulate cell survival and function. ELP-based e-ECM structure mimics many fundamental characteristics of natural ECM including elasticity and structural support through elastin-like domain as well as bioactivity, for example, RGD peptide sequence of fibronectin promotes the cell adhesion. ELP-polyethylene glycol (PEG) hydrogels are composed of a hydrazine- modified ELP (ELP-HYD) and an aldehyde- or benzaldehyde-modified PEG (PEG-ALD or PEG-BZA). Both RGD-ELP/PEG-BZA (2%/2%) and RGD-ELP/PEG-ALD (2%/2%) support high cell-viability. ELP/PEG-ALD/BZA is transparent and is controllable in terms of its mechanical stiffness, stress relaxation rate and bioactivity, which all make this hydrogel system a promising candidate for cell transplantation in tissue regeneration.

37. A High-throughput Screening Approach Aimed Towards Disease Modifying Therapy for Hypertrophic Cardiomyopathy Patients With MYBPC3 Mutations

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Heterozygous truncating Myosin-binding protein C (MYBPC3) mutations are the most common disease causing mutation within hypertrophic cardiomyopathy (HCM), an autosomal dominantly inherited disease. Truncating MYBPC3 mutation transcripts are rapidly cleared from the cell, and decreased total wild-type MYBPC3 protein, or haploinsufficiency is observed. Viral delivery of wild-type MYBPC3 can reverse and prevent disease phenotypes in both animal and cellular models of disease. Thus, one potential therapeutic strategy is to target the cells natural proteastatic mechanisms to restore MYBPC3 homeostasis. Our group has demonstrated the MYBPC3 is degraded in Hsc70-dependent manner via the ubiquitin proteasome system. However, no small molecule is known to specifically increase MYBPC3 protein level to

date. We hypothesize that a cellular based high-throughput screen may enable us to identify small molecules capable of modulating MYBPC3 protein levels. Towards this goal we have developed a novel high-throughput drug screen for small molecules that alter MYBPC3 steady state protein levels within patient-derived induced pluripotent cardiomyocyte cellular model of hypertrophic cardiomyopathy. Initial screening of 42 compounds suggests additional molecular chaperones (specifically Hsp70, Hsp90, VCP) are involved in regulating MYBPC3 homeostasis. We have subsequently completed a larger screen using a library containing 2,4000 FDA approved or known biologically active compounds. Using this library, we have identified 44 validated compounds which significantly alter MYBPC3 protein levels. This work represents an important first step in uncovering natural cellular pathways which regulate MYBPC3 homeostasis. These pathways may influence disease penetrance and offer novel therapeutic targets in the treatment of hypertrophic cardiomyopathy.

38. Clonally-expanding Smooth Muscle Cells Promote Atherosclerosis by Escaping Efferocytosis and Activating the Complement Cascade

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Atherosclerosis is the process underlying heart attack and stroke. Dogma suggests that atherosclerotic plaques expand primarily via the accumulation of cholesterol and inflammatory cells. However, recent evidence suggests that a substantial portion of the plaque may arise from a subset of de-differentiated vascular smooth muscle cells (SMCs) which proliferate in a clonal fashion. Herein we use multi-color lineage tracing models to confirm that the mature SMC can give rise to a 'stem-like' cell that is not only hyperproliferative, but also proinflammatory given its ability to produce complement-dependent anaphylatoxins. Despite being extensively opsonized with pro-phagocytic complement fragments, we find that this cell also escapes immune surveillance, thereby exacerbating its relative survival advantage. Mechanistic studies indicate this phenomenon results from a generalized opsonin-sensing defect acquired by macrophages during polarization. This defect occurs due to the non-canonical upregulation and redistribution of so-called 'don't eat-me' molecules on inflamed phagocytes, which impairs the functionality of their complement receptors and reduces their capacity for programmed cell removal (PrCR). Knockdown or knockout of the responsible anti-phagocytic molecule, CD47, restores the ability of macrophages to sense and clear opsonized targets in vitro, allowing for potent and targeted suppression of clonal SMC expansion in the plaque in vivo. Because integrated clinical and genomic analyses indicate that similar pathways are active in humans with cardiovascular disease, these studies suggest that the 'atherosclerotic stem cell' may represent a new translational target, similar to current oncology efforts directed against the cancer stem cell.

39. Impaired Ventricular Torsion in the SHR

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Torsion is an important component of systolic function, and may contribute up to 40% of ventricular ejection. Treatments aimed at improving torsion may therefore be effective for treating hypertensive heart disease. We investigated torsion in the Spontaneously Hypertensive Rat (SHR) as well as SHRs treated with ACE inhibitors (TSHR) and Wistar Kyoto (WKY) controls. At 3, 14 and 21 months, rats underwent cardiac cine and tagged MRI. Tagged scans were used to track deformation. Torsion was assessed using one apical and one basal slice. Cine scans were used to measure ejection fraction. We characterized torsion for the first time in the SHR, a well-studied animal model of hypertensive heart disease. SHRs that received early ACE inhibitor treatment (3 months) had a significantly greater ejection fraction than their untreated counterparts at 21 months. Stepwise regression revealed that torsion correlated with ejection fraction,

but this correlation was not strong enough to show a significant difference in torsion between treated and untreated rats.

40. Effects of Spaceflight on Human Induced Pluripotent Stem Cell-Derived Cardiomyocyte Structure and Function

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With extended stays aboard the International Space Station (ISS) becoming commonplace, there is a need to better understand the effects of microgravity on cardiac function. We utilized human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) to study the effects of microgravity on cell-level cardiac function and gene expression. The hiPSC-CMs were cultured aboard the ISS for 5.5 weeks and their gene expression, structure, and functions were compared to ground control hiPSC-CMs. Exposure to microgravity on the ISS caused alterations in hiPSC-CM calcium handling. RNA-sequencing analysis demonstrated 2,635 genes were differentially expressed among flight, post-flight, and ground control samples, including genes involved in mitochondrial metabolism. This study represents the first use of hiPSC technology to model the effects of spaceflight on human cardiomyocyte structure and function.

41. E-cigarette Aerosol Elevates Cardiovascular Oxidative Stress in East Asian ALDH2*2 Variant Mice

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Introduction: E-cigarette aerosol contains reactive aldehydes, which induce cellular oxidative stress. Reactive aldehydes are metabolized by a mitochondrial enzyme aldehyde dehydrogenase 2 (ALDH2); a genetic variant known as ALDH2*2 is presented in ~ 560 million people world-wide who cannot efficiently metabolize the reactive aldehydes. Little is known how e-cigarettes, coupled with genetic differences in aldehyde metabolism, affect cardiovascular oxidative stress both at physiological and cellular level.

Methods: E-cigarette Juul aerosol was collected and quantified to measure nicotine and reactive aldehydes levels. Paired age-matched (8 weeks old) male wild type ALDH2 and homozygous ALDH2*2 knock-in mice (identical to human ALDH2*2 variant) were exposed to either Juul or air for 10 days and mice were implanted with EKG telemeters to monitor heart rate daily. Hearts were then subjected to oxidative stress assays.

Results: Juul aerosol contained acetaldehyde (5.3±0.32 ppm), formaldehyde (0.20±0.02 ppm), and acrolein (0.09±0.01 ppm). When rodents were exposed to Juul aerosol, heart rate change was significantly increased in ALDH2*2 mice unlike ALDH2 wild type mice (32.5±32.6 bpm versus -88.2±23.1 bpm, relative to baseline heart rate, respectively, *$p<0.01$, $n=8$). Heart homogenates from ALDH2*2 mice also had higher levels of 4-HNE adducts (1.5-fold), protein carbonyls (2-fold) and lipid peroxides (2-fold) relative to wild type ALDH2 hearts exposed to Juul aerosol (n=4/group).

Conclusions: These data indicate Juul e-cigarette aerosol contains primarily the reactive aldehyde acetaldehyde. A deficiency in aldehyde metabolism by having an ALDH2*2 variant may contribute to increases in heart rate and oxidative stress within the cardiovascular system while smoking e-cigarettes.

42. Comprehensive Investigation of Serum Biomarkers and Their Causal Role in Cardiometabolic Disease

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Circulating biomarkers representing glucose homeostasis, inflammation, lipid metabolism, kidney and liver function have been associated with cardiometabolic traits in observational studies, but the nature of these associations is incompletely understood.

We assessed associations of 27 circulating biomarkers with 7 cardiovascular traits in up to 451,933 participants of the UK

Biobank. Further, we assessed causal relationships of these associations using a two-sample Mendelian randomization (MR) approach combining data from UK Biobank with external GWAS.

In observational analyses, we replicated and extended associations implicated in previous, smaller studies. After multiple-testing correction (alpha=2.6*10-4), we found a total of 15, 9, 21, 22, 26, 24 and 26 biomarkers strongly associated with coronary artery disease (CAD), ischemic stroke, atrial fibrillation, type 2 diabetes (T2D), systolic blood pressure (SBP), body mass index (BMI) and waist-to-hip ratio (WHR); respectively. The MR analyses confirmed strong evidence of previously suggested causal associations for several glucose- and lipid-related biomarkers with T2D and CAD. We detected a protective role of insulin-like growth factor 1 in SBP, and a strong causal association of lipoprotein(a) in CAD development (b, -0.13; per SD change in exposure and outcome and OR, 1.19; P=2.6*10-4 and P=8.2*10-5, respectively). In addition, our results indicated a causal role of increased alanine aminotransferase in the development of T2D and hypertension (OR, 1.73 and b,1.49, per SD change in exposure and outcome; P=1.7*10-6 and P=2.8*10-5).

We confirmed known associations, and reported several novel causal associations providing important insights regarding the etiology of these diseases, which can help accelerate new prevention strategies.

43. Generation of Quiescent Cardiac Fibroblasts From Human Induced Pluripotent Stem Cells for In Vitro Modeling of Cardiac Fibrosis

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Activated fibroblasts are the major cell type that secretes excessive extracellular matrix in response to injury, contributing to pathological fibrosis and leading to organ failure. Effective anti-fibrotic therapeutic solutions, however, are not available due to the poorly defined characteristics and unavailability of tissue-specific fibroblasts. Recent advances in single cell RNA-sequencing fill such gaps of knowledge by enabling delineation of the developmental trajectories and identification of regulatory pathways of tissue-specific fibroblasts among different organs.

This study aims to define the transcriptome profiles of tissue-specific fibroblasts using recently reported mouse single-cell RNA-sequencing atlas and to develop a robust chemically defined protocol to derive cardiac fibroblasts (CFs) from human induced pluripotent stem cells for in vitro modeling of cardiac fibrosis and drug screening.

44. Identification of Causal Antigens in Immune Checkpoint Inhibitor-Induced Myocarditis

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Introduction: Immune checkpoint inhibitors (ICIs) are novel drugs that activate T cell-mediated anti-tumor response leading to improved cancer patient survival. Despite these benefits and their increased usage over the past decade, ICIs can result in side effects including myocarditis ranging from asymptomatic troponin elevation to life-threatening arrhythmias or heart failure. While ICI-induced myocarditis is characterized by myocardial T cell infiltration, the causal mechanisms remain unknown.

Hypothesis: We hypothesize that ICI-induced myocarditis is caused by cardiac-specific auto-antigens triggering clonal expansion of myocardial T-cells, leading to T-cell mediated myocardial damage.

Methods/Results: To understand the ICI-induced inflammatory response, we will perform time-of-flight mass cytometry (CyTOF) to immunophenotype the repertoire of inflammatory cells in Stanford Cancer Center patients diagnosed with ICI-induced myocarditis. Furthermore, we will perform single cell sequencing of T-cell receptors (TCRs) from the blood +/- myocardial-derived T-cell samples. Our preliminary results confirmed the previously reported CD8+ T-cell expansion in the blood and myocardium of myocarditis patients compared with healthy control. By applying Grouping Lymphocyte Interactions by Paratope Hotspots (GLIPH), a novel computational algorithm for predicting the antigen binding pocket structure in T-cell receptors, to our TCR sequencing data, we hope to identify candidate cardiac auto-antigen(s) responsible for this disease.

Conclusion: Myocarditis is a serious and life-threatening complication of ICI treatment. By understanding the unique immune response present during ICI-induced myocarditis and the responsible cardiac auto-antigen(s) involved, we will