Abstracts selected for oral presentation
can be found on pages 1, 9, 10, and 24.

**MLL-Menin inhibition reverses pre-leukemic progenitor self-renewal induced by NPM1 mutations and prevents AML development*

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Abstract:
Recent advances in the study of clonal evolution and step-wise acquisition of mutations during cancer development have revealed that initiating mutations can occur in a pre-malignant state and drastically increase cancer risk. However, options for preventative treatment with targeted therapy that could eradicate pre-malignant clones before they give rise to disease are lacking. Acute myeloid leukemia (AML) is an attractive model system for pre-treatment as it is often preceded by a state of clonal hematopoiesis or myelodysplastic syndrome. Leukemia initiating mutations such as DNMT3a mutations are usually not sufficient for AML development but require co-occurring mutations such as NPM1c. Recent reports suggest that NPM1c mutations can be detected in a premalignant state such as MDS and may act as an indicator of progression to AML. We used a Dnmt3a/Npm1c mutant knock-in mouse model to identify the leukemia initiating population and generate pre-leukemic clones from myeloid progenitor cells. Our data show that Npm1c expression induces a stem cell gene expression program which allows myeloid progenitors to self-renew and engraft long-term before giving rise to leukemia. These pre-leukemic clones could be eradicated by interfering with their aberrant self-renewal properties using the novel orally bioavailable Menin-MLL interaction inhibitor VTP-50469. Furthermore, Menin-MLL inhibition showed high efficacy in eradicating human NPM1c AML cells in PDX models of primary and relapse NPM1c leukemias. These studies suggest it may be possible to prevent AML development in high-risk populations by targeting chromatin associated complexes to reverse preleukemic self-renewal and that the same approaches can be used to treat fully developed disease.

****selected for oral presentation***

In vitro characterization of the human segmentation clock

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Abstract:
The vertebral column is characterized by the periodic arrangement of vertebrae along the anterior-posterior axis. This segmental or metameric organization is established early in embryogenesis when pairs of embryonic segments called somites are rhythmically produced by the presomitic mesoderm (PSM). The tempo of somite formation is controlled by a molecular oscillator known as the segmentation clock. While this oscillator has been well characterized in model organisms, whether a similar oscillator exists in humans remains unknown. As it is not feasible to observe the human segmentation clock in vivo, we have established an in vitro system based on the differentiation of pluripotent stem cells towards PSM fate by dual Wnt activation and BMP inhibition (Chal et al. 2015, 2016). We first verified that in vitro derived cells faithfully recapitulate the segmentation clock by differentiating mouse embryonic stem cells harboring a Hes7 fluorescent reporter and confirming that the resulting PSM-like cells oscillate with normal period (i.e. 2.5 hours). Then, we deployed the same strategy for human induced pluripotent stem cells and observed oscillations of the HES7 cyclic gene with a 5-hour period. Using this system, we found that the human segmentation clock exhibits Wnt, Notch and YAP-dependent oscillations, similar to its mouse counterpart.

We also demonstrate that FGF signaling controls the phase and period of the oscillator. This contrasts with classical segmentation models such as the "Clock and Wavefront" where FGF merely implements a signaling threshold specifying where oscillations stop. Overall, our work identifying the human segmentation clock represents an important breakthrough for human developmental biology.

Genome scale in vivo CRISPR screen identifies RNLS as a target for beta cell protection in type 1 diabetes

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Abstract:
Type 1 diabetes (T1D) is caused by the autoimmune destruction of pancreatic beta cells. Pluripotent stem cells can now be differentiated into beta cells, raising the prospect of a cell replacement therapy for T1D. However, autoimmunity would rapidly destroy newly transplanted beta cells. Using a genome-scale CRISPR screen in a mouse model for T1D, we discovered that deleting RNLS, a GWAS candidate gene for T1D, made beta cells resistant to autoimmune killing. Structure-based modeling identified the FDA-approved drug pargyline as a potential RNLS inhibitor. Oral pargyline treatment protected transplanted beta cells in diabetic mice, leading to disease reversal. Further, pargyline could prevent or delay diabetes onset in several mouse models for T1D. Our results identify RNLS as a modifier of beta cell vulnerability and as a potential therapeutic target to avert beta cell loss in T1D.

Stem Cell-derived Neurons Provide the Missing Link between ALS Pathology and Motor Neuropathy

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Abstract:
Aggregation of the RNA-binding protein TDP-43 in vulnerable neurons is a diagnostic pathology for most patients with ALS. Furthermore, mutations in the gene TARDBP, which encodes for TDP-43, is a cause of familial ALS. Although proposed that these genetic and pathological perturbations disrupt RNA metabolism, the identity of the RNAs regulated by TDP-43 in human neurons remains elusive. Here, we identified using RNA-seq transcripts whose abundance in purified human stem cell-derived motor neurons (hMNs) were sensitive to TDP-43 depletion. We discovered that transcript levels of STMN2, a regulator of microtubule stability and neurite extension, were reproducibly and sharply decreased. This reduction was also found in hMNs differentiated from patient-derived iPS cells with TDP-43 mutations. STMN2 loss was due to altered splicing, which is functionally important, as we demonstrate STMN2 is necessary for normal axonal outgrowth and regeneration. The in vivo validation of discoveries from stem cell-based models of ALS is a critical test of their relevance to disease mechanisms. To this end, we used ALS patient spinal cord tissues to provide in vivo evidence corroborating our disease modeling studies that TDP-43 dysregulation alters the expression of STMN2 through altered splicing. We further leveraged this molecular information of altered STMN2 splicing to develop a potential ALS biomarker assay, and we have identified compounds that can correct this splicing defect that could serve as an ALS therapeutic. In conclusion, human stem cell-based models can be used to discover unique aspects of human biology underlying disease pathomechanisms and can illuminate potential therapeutic targets.

From bedside to the bench: a novel human homozygous IGF1R mutation is causal of abnormal skeletal acquisition

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Abstract:
Introduction: The fundamental importance of the insulin-like growth factor-1 (IGF-1)-IGF1R (IGF-I receptor) system in skeletal acquisition and bone mineral density (BMD) accretion during developmental growth and aging is supported by mouse studies and limited human case reports. We now report two siblings, born IUGR to consanguineous parents, had severe post-natal growth retardation with height SDS of -6.2 (P1, female, 9.9yrs) and -4.5 (P2, male, 7.1 yrs). Co-morbidities included microcephaly, dysmorphic features, and a markedly reduced BMD of -6.9 SD (P1) and -3.7 SD (P2). Whole exome sequencing identified a novel homozygous IGF1Rc.2132_2143del, which resulted in an IGF1R variant that was appropriately expressed and localized, but IGF-I responsiveness was significantly blunted.

Objectives: To assess the impact of a human homozygous IGF1R p.Ala711_Glu714del in-frame mutation on skeletal accretion in a knock-in mouse model.

Methods: A viable knock-in mouse model of the human mutation (IGF-1RKI) provided in vivo evidence that the private IGF1R mutation was the cause for the loss of bone integrity and impaired growth. Igf1r c.2134_2145del (p,Ala712_Glu715del) in C57BL/6J background was generated by gene-editing with CRISPR/Cas9 methodology and mice bred for heterozygous and homozygous knock-in genotypes. Bone morphology and BMD were analyzed by high-resolution micro-computed tomography (mCT), mechanical properties were studied by 3-point bending and micro-indentation assays.

Results: Male and female IGF-1RKI homozygous mice, born IUGR, displayed significant post-natal growth retardation. Reduced ossification of the axial and appendicular skeleton detected in one-day-old heterozygous mice was more severe in the homozygous mice. Both sexes showed 45% reductions in femur mid-diaphysial total cross-sectional area and bone area, and a 30% decrease in cortical bone thickness that resulted in reductions in polar moment of inertia and mechanical strength at 8 and 16 weeks of age. Despite the severely compromised morphology of the femur in the homozygous mice, BMD of the cortical bone compartment was surprisingly similar to control mice. In contrast, the trabecular bone at the femur distal metaphysis of homozygous males showed 40% and 30% reductions in bone volume/total volume (BV/TV) and BMD, respectively, while homozygous females showed minor reductions in both BV/TV and BMD at 8 and 16 weeks.

Conclusion: The skeletal phenotype of our Igf1r knock-in mouse indicates severe delays in accretion of BMD and impaired trabecular bone metabolism, thus mimicking the loss of BMD in our patients. Future studies of osteoprogenitors number and function will unravel the mechanisms of how this specific natural mutation in IGF1R impaired skeletal development and contributes to the severe post-natal retardation observed in the patients.

A self-amplifying, self-propagating loop of Yap and Shh drives formation and expansion of heterotopic ossification

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Abstract:
Heterotopic ossification (HO) refers to extraskeletal pathological bone formation that occurs as a common complication after injury or as a manifestation of particular genetic disorders. However, limited by poor understanding of the underlying cellular and molecular mechanisms, there is currently no effective treatment and surgical eviction often results in recurrence. We have found that in both genetic and injury-induced HO, activated ectopic Shh expression drives HO progression by forming a positive feedback loop that results in non-cell-autonomous and self-propagation of osteoblast differentiation of wild type cells. In mouse models of progressive osseous heteroplasia (POH), a human disease caused by null mutations in GNAS that encodes Gαs, we found that progressively expanded ectopic bone was formed by progressively recruited wild type cells. Mechanistically, Gnas-/- mesenchymal cells differentiate into osteoblasts and recruit wild-type cells to form bone by activating Yap, a transcription factor that regulates Shh expression and secretion. Secreted Shh further induces Yap activation, Shh expression and osteoblast differentiation in surrounding wild type cells. This self-propagating positive feedback loop is both necessary and sufficient for ectopic bone formation and expansion and can act independently of Gαs. Importantly, Yap and Shh activation was also found in fibrodysplasia ossificans progressive (FOP), Achilles tendon puncture (ATP)-induced HO mouse models and in human HO samples. Genetic removal of Yap and Shh abolished HO not only in POH, but also in FOP and acquired HO mouse models. We therefore identify a Yap-Shh positive feedback loop as a common cellular and molecular mechanism underlying HO initiation and expansion. Our work highlights the importance of rare genetic disease studies in identifying a shared core pathological mechanism underlying a class of diseases. As Shh is not required for normal bone formation, our results further suggest that Shh is a promising new therapeutic target for HO without affecting the normal bone.

Mesenchymal Alterations Underly Lung Defects Associated with Bronchopulmonary Dysplasia

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Abstract:
Bronchopulmonary dysplasia (BPD) is a lung disease characterized by chronic respiratory impairment. The etiology of BPD is rooted in the high level of oxygen that preterm neonates are exposed to in order to maintain an acceptable level of oxygen in the bloodstream. BPD is a blockage of the normal lung development that results in the overall simplification of the alveolar architecture.

We hypothesize that in BPD, pathological alterations of the lung microenvironment lead to aberrant signaling to lung epithelial progenitor cells, ultimately resulting in dysfunctional development. Using a BPD mouse model, we observed that lung progenitor cells from diseased pups exhibit better proliferation and differentiation abilities specifically directed towards alveolar differentiation. Conversely, normal lung progenitors have decreased ability to differentiate into alveolar organoids when co-cultured with mesenchymal cells derived from the murine model of BPD. These findings argue that the mesenchymal compartments might be responsible for the defective alveolar development while the pool of epithelial stem cells in the distal lung of a BPD mouse model are healthy and accumulate in an "unspent" state that could be therapeutically tapped.

To further pinpoint the mesenchymal cell type responsible for this disease we conducted a single cell sequencing experiment to compare the stromal populations of healthy mouse pups vs BPD. This approach identified two distinct clusters that are uniquely affected in the BPD mouse model. We are currently examining the molecular differences of the diseased populations in order to define the aberrant signaling nodes that underlie BPD at a molecular level.

**Organoid Maturation by Circadian Entrainment*

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Abstract:
Stem cell-derived organoids that recapitulate endogenous physiology could transform disease research and therapy, yet most methods yield products that function like fetal, not adult tissues. Organoids are typically grown in constant environments, whereas our tissues turn functionally mature in the presence of daily physiological rhythms. To better understand maturation-driving mechanisms, we studied epigenome dynamics during the stepwise formation of pancreatic islet organoids from human pluripotent stem cells. We devised tools to purify intermediates across islet lineage progression, and profiled their DNA methylation, accessibility, and histone modification changes. Unexpectedly, we found BMAL1/CLOCK footprints enriched at genomic sites activated as organoids gain glucose-responsive insulin secretion. Moreover, we uncovered transcriptional loops between circadian effectors DEC1/2 and factors linked to mature insulin responses. This suggested circadian clocks may foster organoid maturation. Indeed, entrainment to 12h feeding-fasting cycles induces organoid clocks, eliciting pulsatile synthesis of energy metabolism and insulin secretion effectors, including antiphasic insulin-glucagon pulses. This triggers metabolic rhythms and cyclic insulin responses with higher glucose threshold and single-cell secretion, a hallmark of islet maturity. Clock-entrained organoids gain stable epigenetic changes at genes enabling mature insulin responses, and function within days of transplant. Clock control of islet maturity effectors depends on Dec2 binding their promoters/enhancers, and Dec2 loss causes insulin responses with low secretion and glucose threshold. Accordingly, β cell-specific Dec2 deletion renders mice diabetic, despite intact islet mass, insulin content, and Bmal1/Clock function. This reveals a mechanism linking circadian rhythms to metabolic maturity, which can be harnessed to further maturation of stem cell-derived organoids.

****selected for oral presentation***

**Engineering gene therapies of aging*

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Abstract:
Over the past decades, research into aging has uncovered numerous genes whose modulation protects against age-related diseases and extends healthy lifespan. However, aging research remains slow and laborious due to the need to generate and age transgenic animals.

Furthermore, most discoveries cannot easily be translated to the clinic. These problems are major but potentially solvable through the use of gene delivery technologies, to study effects of genes directly in aged mice, and to translate findings directly to patients via gene therapies. However, no known gene delivery technology is able to reach sufficiently wide area of the body while allowing uniform and long-term expression to enable this. To solve these problems, we have developed DAEUS - a high-efficiency body-wide gene delivery system based on AAV. Unlike other gene delivery systems, DAEUS achieves uniform, long-term overexpression of transgenes in over 70% of cells across multiple tissues in aged mice and allows expression to be tuned in different tissues. Using DAEUS, we achieve systemic overexpression of three geroprotective genes in aged mice. Furthermore, in a mouse model of Wolfram Syndrome II (a human and mouse progeria caused by loss of Cisd2), delivery of Cisd2 using DAEUS restores Cisd2 expression systemically to wild-type levels and protects against development of progeria in multiple cohorts. Surprisingly, DAEUS-Cisd2 gene therapy also precludes death and rejuvenates multiple tissues in a Wolfram Syndrome mouse with advanced disease. In summary, gene delivery using DAEUS holds promise to speed up aging research and streamline translation to the clinic.

selected for oral presentation

A Human In Vitro Model for Type-1 Diabetes Reveals Gene Editing Targets for Immune Protection of Stem Cell-derived Beta Cells

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Abstract:
Type 1 diabetes (T1D) is an autoimmune disorder leading to the destruction of insulin-producing β-cells in the pancreas. Despite recent scientific advances, questions remain regarding the initial trigger and the downstream mechanisms of disease progression. Human induced pluripotent stem cells (hiPSCs) provide new opportunities for cell replacement therapy of T1D. Therapeutic quantities of human stem cell-derived β-cells (SC-β) can be attained in vitro following a stepwise differentiation protocol. Yet, preventing immune rejection of grafted cells, without the use of life-long immunosuppressants, remains a major challenge. Using T1D patients' hiPSC derived β-cells (iPSC-β), we developed a human in vitro platform in an autologous setting that recapitulates aspects of the effector/target interactions in an autoimmune response. A donor-matched β-cell-specific response was achieved by co-cultures with perihelial blood mononuclear cells (PBMCs) derived from the same donors' blood. We performed a droplet based single-cell RNA sequencing (scRNA-seq) of T1D iPSC-β co-cultured with their autologous PBMCs. scRNA-seq data analysis of co-cultured cell populations identified upregulated genes that contribute to the inflammatory microenvironment of a T1D pancreatic islet. Subsequent co-culture experiments have shown that CRISPR-depletion of such genes in SC-β, can reduce activation of T-cells and increase β-cell survival. These results provide insights into the nature of immune destruction of β-cells during T1D and suggest a path to prevent it in cell replacement approaches.

Deep learning-based analysis of the differentiation in 3D retinal organoids

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Abstract:
The three-dimensional, "organoid" approach for the differentiation of pluripotent stem cells into retinal and other neural tissues has become a major in vitro strategy to recapitulate development. In part, that success is owed to the availability of fluorescent reporter cell lines that allow to quickly and non-invasively assess differentiation. However, design and validation of reporter cell lines with genetic background of interest is time consuming and not compatible with clinical application. We hypothesized that basic contrast brightfield images contain sufficient information on the tissue specification and it is possible to extract this data using convolutional neural networks (CNN).

Retina-specific reporter Rx-GFP mouse embryonic reporter stem cells have been used for the differentiation experiments. The brightfield (BF) images of organoids have been taken on day 6 and fluorescent on day 9. To train the CNN we utilized a transfer learning approach: ImageNet pre-trained ResNet50v2 CNN had been trained on 3 000 labeled BF images, divided into two categories (retina and non-retina), based on the fluorescent reporter gene expression. A comparison of CNN with the human-based classifier showed that the CNN algorithm performs better than the expert in predicting organoid fate: 0.84% vs 0.67 ± 0.06% of correct predictions respectively, confirming our original hypothesis. Overall, we have demonstrated that computer algorithm can successfully predict retinal differentiation in organoids before the onset of reporter gene expression, which forms the basis for universal, non-invasive, scalable and rapid approach to assess the state of the cell and forecast its fate.

Targeting immune checkpoint as a novel strategy for anti-aging intervention

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Abstract:
Cellular senescence is one of the hallmarks of aging. Senescence triggers chronic inflammation in aged tissues, which plays a causative role in many age-associated diseases.

Senescent cells accumulate in aged tissues and shorten healthy lifespan in mice. Clearance of senescent cells delays age-associated disorders in mouse models and preclinical studies of a variety of diseases. This instigates the efforts to develop senolytic drugs to remove senescent cells from aged tissues. However, senolytics have documented side effects and toxicity to healthy cells, limiting their applications in the elderly.

Senescent cells are subjected to immune surveillance. A broad spectrum of immune cells can be recruited to tissues to mediate clearance of senescent cells. However, senescent cells accumulate in aged tissues, without being cleared by the immune system. The underlying mechanisms for the failed immunosurveillance is unknown.

My preliminary results indicate that senescent cells upregulate PD-L1, a ligand for PD-1, which is a major inhibitory receptor on immune cells. This upregulation is dependent on cGAS-STING and STAT activity. Inhibition of PD-L1/PD-1 can incur the hair-regrowth in aged mice. I hypothesize that senescence employs a similar mechanism as cancer to inhibit the immune system, and that targeting the PD-L1 pathway in senescent cells can restore immune-mediated clearance.

I will conduct drug and genetic screen to identify novel agents that can downregulate PD-L1 on senescent cells to promote immunosurveillance. This will introduce a pioneering concept to target senescent cells, with potential of developing novel therapies to delay aging and ameliorate age-associated functional decline.

Efficient, Specific and Universal Therapeutic Gene Editing of ELANE For Severe Congenital Neutropenia in Human Hematopoietic Stem Cells

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Abstract:
Severe congenital neutropenia (SCN) is a life-threatening disorder most often caused by dominant mutations of ELANE (encoding neutrophil elastase, NE) that interfere with normal neutrophil maturation. Granulocyte colony stimulating factor (G-CSF) pharmacotherapy alleviates neutropenia but unmasks and accelerates an elevated lifelong risk of myeloid leukemia. Here we perform a pooled CRISPR screen in human hematopoietic stem and progenitor cells (HSPCs) to correlate ELANE mutations with neutrophil maturation potential. Highly efficient ELANE gene editing of early exons elicits nonsense-mediated decay (NMD) and overcomes neutrophil maturation arrest in HSPCs from ELANE mutant SCN patients. Conversely, terminal exon ELANE frameshift alleles that mimic SCN-associated mutations escape NMD and recapitulate neutrophil maturation arrest in healthy donor cells. In mouse xenografts, human HSPCs with early exon gene edits demonstrate normal hematopoietic engraftment function whereas HSPCs with late exon gene edits reproduce selective neutrophil maturation arrest in vivo, establishing the first animal model of ELANE mutant SCN. Surprisingly, only -1 frame indels impede neutrophil maturation, while -2 frame late exon indels repress translation and support neutrophil maturation. Gene editing of primary hematopoietic cells represents a universal therapeutic approach to ELANE mutant neutropenia compatible with HSC function and allows faithful identification of variant pathogenicity that clarifies the molecular pathogenesis of SCN.

Transcriptional regulation of mitochondrial metabolism by TIF1γ drives erythroid progenitor differentiation

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Abstract:
Understanding in-vivo mechanisms of hematopoiesis is critical for developing directed blood differentiation approaches. Zebrafish moonshine (mon) mutant embryos defective for the conserved transcriptional intermediary factor 1 gamma (tif1λ) do not specify enough erythroid progenitors. To elucidate the TIF1λ -mediated mechanisms in erythroid differentiation, we performed a chemical suppressor screen and identified inhibitors of the essential mitochondrial pyrimidine synthesis enzyme dihydroorotate dehydrogenase (DHODH). Leflunomide as well as the structurally unrelated DHODH inhibitor brequinar rescue the formation of erythroid progenitors in 61% (38/62) and 68% (50/74) of mon embryos, respectively. In-vivo metabolomics analyses identified nucleotide metabolism as the most significantly altered process in mon mutants, with elevated levels of uridine monophosphate. This increase is functionally linked to a reduced oxygen consumption rate. DHODH is the only enzyme of the pyrimidine de novo synthesis pathway located on the inner mitochondrial membrane and its activity is coupled to that of the electron transport chain (ETC) via coenzyme Q (CoQ). Rotenone, a potent ETC complex I inhibitor reverses the rescue of the erythroid progenitor defect by DHODH inhibition in mon embryos. Through parallel genome-wide transcriptome and chromatin immunoprecipitation analyses, we found that genes encoding CoQ metabolic enzymes are direct TIF1λ targets.

Treatment with the CoQ analog decylubiquinone results in rescue of erythroid progenitors in 26% (33/126) of mon embryos. Our work highlights the importance of transcription regulatory processes for tuning metabolism to drive cell fate decisions during lineage differentiation and could have therapeutic implications for blood diseases.

Zebrafish chemical compound screen uncovers inducers of skeletal muscle engraftment across species

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Abstract:
Genetic muscle disorders compromise muscle function through degeneration of muscle fibers, increased inflammation and impaired muscle regeneration. Transplantation of genetically normal muscle progenitors could be an approach to rescue muscle wasting and boost repair; however, this approach has shown limited utility thus far due to the typically poor engraftment efficiency of cultured progenitors. To define regulators of muscle engraftment that could be targeted to improve transplantation outcomes, we developed a novel cross-species screening platform, employing zebrafish and mouse, to discover chemical compounds that promote muscle progenitor engraftment in vivo. Muscle cells derived from zebrafish blastomeres were treated for 4 hours with biomolecules and transplanted into the flanks of adult zebrafish (n=15/ biomolecule). We focused our screening on a well-annotated library of 230 lipids, since lipids are known to enhance cell migration and regulate the homeostasis and regenerative function of adult tissue stem cells. Using limit-dilution assays, potential "hits" from our primary screen were identified and re-evaluated in replicate transplantation experiments. We discovered two lipids that promote zebrafish muscle progenitor cell engraftment in vivo: lysophosphatidic acid (LPA) and niflumic acid (NFA). Using bioluminescence imaging, we further ascertained that both NFA and LPA enhance muscle stem cell (satellite cell) engraftment in mouse as well (mean BLI radiance ± SEM- NFA: 27.2E+6 ± 6.8 E+6 p/s; LPA: 25.6 E+6 ±4.4 E+6 p/s; vehicle-treated cells: 8.2 E+6 ± 1.4 E+6 p/s; n=15, 1-way ANOVA, p ? 0.05), indicating conservation of the pro-myogenic activities of these compounds across vertebrate species. Studies in sapje-like (dystrophin mutant) fish transplanted with NFA-treated or LPA-treated cells showed higher engraftment efficiency, significantly better swimming performance and greater ability to swim

against a water current, compared to fish engrafted with vehicle-treated control cells. Mechanistically, the pro-myogenic activities of LPA and NFA appear to be associated with increased cytoplasmic Ca2+ and down-regulation of muscle development genes. RNA sequencing analysis also revealed upregulation of myoblast fusion regulating genes, including myomaker (Tmem8c) and Ccl8, in LPA-treated satellite cells. In summary, successful application of this cross-species approach has uncovered evolutionarily conserved pathways regulating muscle regeneration, suggesting new potential opportunities for treating muscle disease by enhancing myogenic contributions of transplanted muscle progenitors.

Extracellular Vesicles Induce Mesenchymal Transition and Therapeutic Resistance in Glioblastomas through NF-κB/STAT3 Signaling

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Abstract:
Glioblastoma (GBM) is the most common primary malignant brain tumor and despite optimal treatment, long-term survival remains uncommon. GBM can be divided into three different molecular subtypes, each varying in aggressiveness and treatment resistance. Interestingly, recent evidence shows plasticity between these subtypes in which the proneural (PN) glioma stem-like cells undergo transition into the more aggressive mesenchymal (MES) subtype, resulting in therapeutic resistance.

Extracellular vesicles (EVs) are a heterogeneous group of membrane-limited vesicles originating from the plasma membrane or endosome. They are secreted by nearly every cell and have been shown to play a key role in GBM progression by acting as multifunctional signaling complexes.

Here, we show that EVs derived from MES cells modulate PN cells to increase proliferation, migration potential, stemness, invasiveness, aggressiveness, and therapeutic resistance by inducing mesenchymal transition through NF-κB/STAT3 signaling. Our findings could potentially help explore new treatment strategies targeting intratumoral EV crosstalk and further suggest that EVs may play a role in mesenchymal transition of other tumor types.

Organoid-derived Retinal Ganglion Cells are Suitable for Cell Replacement in the Murine Retina

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Abstract:
Cell replacement promises to restore previously lost vision for patients with advanced Glaucoma and other optic neuropathies. In contrast to neuroprotective treatments and gene therapy approaches, which may halt visual degeneration by preserving host neurons, cell replacement aims to reverse degenerative damage by addition of new donor cells. To enable successful cell replacement, retinal neurons need to be manufactured on a clinically relevant scale from renewable cell sources. Over the past years, our laboratory and others have shown that retinal neurons can be differentiated in-vitro from ES/iPS cells within 3-dimensional retinal organoids. Our work highlights that within three weeks of culture retinal ganglion cells (RGCs) derived from Thy1-GFP miPSCs exhibit functional, morphological, and molecular characteristics reflective of diverse RGC subclasses observed in-vivo. When cultured with slow-release growth factors BDNF/GDNF (PODS) organoids yielded up to 5% of RGCs, illustrating the protocols potential for translation to a clinical manufacture scale.

Following isolation with magnetic microbeads, RGCs were formulated at 20,000 cells in 2ul and transplanted into the vitreous of healthy mouse pups, adults as well as mouse models of NMDA and microbead-induced RGC loss, mimicking glaucomatous RGC degeneration. Across all conditions, transplant success exceeded 50%, with healthy hosts retaining donor RGCs in 80-100% of cases. Modulation of host micro-environment via PODS co-delivery proofed essential to transplant success. Donor RGCs were confirmed to survive up to 12 months within host retinas and were observed to extend axonal projections into the host optic nerve, arguing for their potential to rewire the retina-brain neurocircuit.

3D-printed ABCB5+ dermal stem cells for the treatment of limbal stem cell deficiency

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Abstract:
Limbal stem cells (LSC) residing in the limbus continually repopulate the clear corneal epithelium at the front of the eye. Limbal stem cell deficiency (LSCD) occurs when these LSC are damaged (due to burns, infections, or other trauma) or missing (due to genetic conditions). Patients with LSCD are unable to regenerate the corneal epithelium, resulting in blindness due to conjunctival in-growth and neovascularization. Unilateral LSCD can be treated by transplantation of autologous limbal tissue or ex vivo expanded limbal cells from the unaffected eye, however, patients with bilateral LSCD have no source of autologous LSC. Treatment with allogeneic limbal tissue from cadaveric donors has a less favorable outcome and typically provides only temporary relief without long-term restoration of the corneal epithelium.

Our lab discovered that ABCB5 is a specific marker of LSC and that ABCB5+ LSC can restore the corneal epithelium in a LSCD mouse model. We also discovered that ABCB5 marks dermal stem cells (DSC) and performed a preliminary pilot study demonstrating the ability of ABCB5+ DSC to express the corneal epithelial markers PAX6 and KRT12 in culture. Furthermore, transplantation of human ABCB5+ DSC in a LSCD mouse model led to the formation of clear corneas. We are currently using 3D bioprinting technology to develop a stem cell-based corneal-limbal bio-prosthesis that can be implanted into wounded corneas. The use of ABCB5+ DSCs as an alternative autologous source of LSC and 3D-printing to recreate the LSC niche has the potential to greatly improve therapy for patients with bilateral LSCD.

Understanding SHIP1 Expression and Function in the Human Brain

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Abstract:
Alzheimer's disease (AD) is a progressive neurodegenerative disease that affects memory and other cognitive functions. Recent studies suggest that the resident immune cells of the brain, microglia, may play a more active role in neurodegeneration either through microglia engulfing synapses or from the secretion of pro-inflammatory cytokines as a result of microglia responding to elevated Aß. Thus, understanding the regulation of microglial activation is critical towards illuminating the role of microglia in the adult human brain and in the disease state. SHIP1, or SH2-domain containing inositol polyphosphate 5-phosphatase 1, is a protein that has been identified in GWAS studies to be associated with late-onset forms of Alzheimer's disease. In peripheral immune cells, SHIP1 has been shown to dephosphorylate phosphatidylinositol-3,4,5-trisphosphate [PI(3,4,5)P3] to generate phosphatidylinositol-3,4-bisphosphate [PI(3,4)P2]. However, the expression and function of SHIP1 is largely unknown in the brain.

Elucidating the role of SHIP1 in microglia would not only offer more insight into the dynamic regulation of microglia in the non-disease state, but also may advance our understanding of AD pathogenesis. Through RNA sequencing and Western blotting, we have demonstrated that SHIP1 expression is restricted to human microglia in both postmortem brain and human iPSC-derivatives. Further, immunostaining in human brain tissue has confirmed that SHIP1 expression is limited to human microglia. Finally, we have generated human iPSC-derived microglial models of SHIP1 loss-of-function and are now characterizing how reduction in SHIP1 activity affects microglial biology.

Vascular smooth muscle origin of cold-induced brown adipocytes

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Abstract:
Brown adipose tissue (BAT) is a specialized type of adipose tissue which promotes energy expenditure. Considering the capacity of BAT for energy dissipation and its role in glucose and lipid metabolism, increasing BAT mass or activity could potentially be utilized to combat obesity and its related metabolic disorders. Adipose tissue is composed of adipocytes, adipocyte progenitors, vascular cells, immune cells, and neurons. Although adipocytes play the major role in maintaining energy balance, other cell types form the adipocyte niche and regulate adipose tissue function. Cold exposure increases BAT mass through de novo recruitment of brown adipocytes to enable maximal thermogenic activity. However, the source of cold-induced brown adipocytes and the molecular mechanism regulating BAT expansion is not known. To determine the cellular origin of brown adipocytes, we performed single-cell RNA-sequencing of BAT from mice housed at different temperatures. Our analysis identified two distinct types of adipocyte progenitors: the previously reported Sca1pos Pdgfrapos mesenchymal progenitors and a new vascular smooth muscle-derived adipocyte progenitor cell (SM-APC) population that contribute to de novo recruitment of thermogenic adipocytes in cold. Using flow cytometry and lineage tracing, we demonstrated that the SM-APCs were indeed distinct from the Pdgfrapos progenitors and could contribute to thermogenic brown adipocytes in response to cold challenge. Together, these findings reveal a novel cellular origin of thermogenic adipocytes and suggest a new model for the development of BAT that could be critical in designing strategies to increase the number of brown adipocytes in humans.

**3D Bioprinted niche topography defines epigenetic and genetic landscapes of wound healing pathways in human epidermal stem cells*

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Abstract:
Rete ridges are epidermal downgrowths that form niches containing interfollicular stem cells important in wound healing, skin regeneration and homeostasis. To investigate the mechanobiological impact of niche topography on the epigenetic/genetic profiles of epidermal stem cells, 3D-bioprinted bovine collagen scaffolds were imprinted using custom stereolithography-generated stamps to replicate anatomically correct rete ridges. Human keratinocytic stem cells (hKSCs) were seeded onto un-stamped flat control surfaces or into the "rete ridge" concavities, where they maintained expression of K15, a key marker of interfollicular epidermal cells. RNA-seq analysis revealed a complex transcriptional profile in "rete ridge" hKSCs, including significant (1) downregulation of skin stem cell markers such as ITGA6 but with upregulation of K15 and Wnt pathway members implicated in stem cell maintenance, (2) upregulation of over 20 key epidermal differentiation complex (EDC) genes, and (3) differential regulation of pathways associated with wounding and inflammatory responses. ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing) analysis disclosed globally increased chromatin accessibility in "rete ridge" hKSCs, with non-uniform degrees of peak enrichment across genetic sites. Preliminary integration of these two analyses identified a select group of upregulated genes associated with ATAC-seq peaks localized to the promoter region (<500bp of TSS), including critical EDC genes, DUOX1 and DUOXA2, which have been implicated in wound healing and repair mechanisms. These aggregate data suggest that niche topography alone is capable of defining genetic and epigenetic identity of its resident epidermal stem cells, with changes potentially reflective of an "activated" stem cell phenotype poised for response to skin injury.

selected for oral presentation

Single cell analysis of JAK2-mutant myeloproliferative neoplasms

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DFCI Data Sciences (MN, JE, SL, FM, SH), DFCI Medical Oncology (CR, BK, AM), EMBL-EBI (MK, IC), MGH Hematology/Oncology (GH)

Abstract:
Myeloproliferative neoplasms (MPNs) are a group of malignant blood disorders characterized by overproduction of red blood cells or platelets. This clinical phenotype is often caused by clonal expansion of hematopoietic stem and progenitor cells (HSPCs) that have a somatic gain-of-function mutation in the JAK2 gene, leading to constitutive kinase signaling through erythropoietin, thrombopoietin, and other cytokine receptors. To investigate the effects of the most common JAK2 mutation (JAK2 V617F) on HSPC behavior in clinical disease, we performed single-cell genomic and transcriptomic profiling on CD34+ bone marrow HSPCs from seven MPN patients. Our results from whole genome sequencing suggest that mutations in JAK2 occur in the bone marrow decades before diagnosis, and that during this period without clinical disease the JAK2-mutant clone expands and has a fitness advantage over WT HSCs. scRNA-seq of CD34+ HSPCs from MPN patients with joint single-cell genotyping of the JAK2 mutation locus shows that JAK2-mutant cells have an erythroid-megakaryocyte fate bias relative to WT HSPCs.

However, JAK2-mutant and WT cells are otherwise transcriptomically similar, except for notable upregulation of the leptin receptor and some immune-related genes in JAK2-mutant cells. Therefore, our results suggest that in MPN patients, mutations in JAK2 occur at the highest levels of the hematopoietic differentiation hierarchy relatively early in life and accelerate erythroid-megakaryocyte fate commitment in these cells.

Chemical genetics screen identifies vinca alkaloids as promoters of platelet formation

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Abstract:
Platelet function is critical to hemostasis, and millions of platelet units are perfused every year to manage thrombocytopenia. Supply shortages, functional variability, risk of contamination, as well as histo-incompatibility issues are inherent to conventional donor platelets, but these limitations could ultimately be overcome by transfusion of human induced pluripotent stem cell (hiPSC)-derived platelets engineered to lack problematic antigens. However, in vitro megakaryocyte (MK) differentiation and platelet production systems generally suffer from low yields. We developed imaging platforms for assessing maturation phenotypes in hiPSC-derived and conditionally immortalized megakaryocytic cells (imMKCLs). Single-cell time-course imaging revealed that imMKCLs maturation is heterogeneous, with only a minority producing large quantities of platelet-like particles.

Through a 1536 well-plate based chemical genetics screen we identified vincristine and vinblastine as compounds that promote maturation and the formation of pro-platelet-like extensions by most imMKCLs. Vinca alkaloids have been associated with thrombocytosis, but they have not previously been shown to promote MK maturation. MK maturation and platelet production yields could also be augmented by addition of CCT137690, an Aurora Kinase inhibitor that promotes polyploidization and that synergizes with vincristine in boosting imMKCL terminal differentiation and platelet production. Application of both compounds in a vertical wheel bioreactor system allowed production of >90 platelets/imMKCL or >10e11 (~ 1 unit) platelets per 10-liter culture, a ~22.5 fold improvement over our starting conditions. The bioreactor-produced platelets are structurally and functionally similar to conventional human donor platelets as judged by size, morphology, ultrastructure, resting and agonist stimulated

surface expression of relevant biomarkers as well as platelet aggregation. Mouse in vivo studies demonstrate that transfused bioreactor-produced human platelets participate in thrombus formation and reduce bleeding times in vascular injury models.