1. Normal and HCC cell spreading dynamics in response to viscoelasticity

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The cellular microenvironment plays a critical role in cell differentiation, proliferation, migration, and homeostasis. Recent studies show the importance of substrate viscosity in determining cellular function. Here, we study the mechanoresponse of normal hepatocytes and hepatocellular carcinoma cells (HCC) to elastic and viscoelastic substrates. This was accomplished using the Huh7 cell line, which was derived from a human liver tumor and primary human hepatocytes (PHH). Unlike PHH and fibroblasts, which respond to viscoelastic substrates by reducing spread area and actin bundle assembly compared to purely elastic substrates of the same stiffness, Huh7 cells spread faster on viscoelastic substrates than on purely elastic substrates. The steady state spread areas of Huh7 cells are larger on viscoelastic substrates, whereas the opposite effect occurs with PHH cells. The viscoelasticity of the microenvironment also promotes motility and multiple long protrusions in Huh7 cells. Pharmacologic disruption of actin assembly makes cells unable to spread on either elastic or viscoelastic substrates. In contrast, upon vimentin perturbation, cells still spread to a limited degree on elastic substrates, but are unable to spread on viscoelastic substrates. The time evolution of cell traction force shows large changes in contractile energy on elastic substrates, but the total force generation is the same on both these substrates after a 4 hr time period. Our data suggest that stress relaxation time scales of the viscoelastic substrate regulate cell dynamics and traction force generation, indicating different binding-unbinding rates of the proteins that form cell attachment sites in HCC cells and normal hepatocytes. These results suggest that liver cancer cells may have different characteristic lifetimes of binding to the substrate in comparison with normal cells, leading to different cell spreading and motility within the diseased tissue.

2. PAX8 drives ovarian cancer angiogenesis through interaction with SOX17

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A major problem in the treatment of ovarian cancer is the heterogeneity among ovarian tumors. DNA and RNA sequencing studies have demonstrated both intertumoral and intratumoral genetic variation. Despite efforts to elucidate common signaling pathways among various ovarian cancer subtypes, few have led to meaningful patient stratification or individualized targeted molecular therapies. A theoretical new approach to the treatment of ovarian cancer is to target the signaling pathways that are essential to the development of the progenitor cells - the secretory epithelium of the fallopian tube. PAX8, a transcription factor that identifies and sustains ovarian cancer, is also the main regulator of fallopian tube development. We investigated whether blocking the ability of PAX8 to influence its gene targets, either by interrupting PAX8 protein-protein interactions or by inhibiting the products of PAX8-driven signaling, has the potential to eliminate the primary growth stimulus for ovarian tumors. Herein, we have identified SOX17 as a bona fide PAX8-interacting partner and elucidated their collaborative impact on ovarian cancer. We observed that PAX8 and SOX17 are master regulators of ovarian cancer identity, as both were found binding in super-enhancer regions regulating most of same set of genes. Ontology analysis after PAX8 or SOX17 loss showed alteration in three main pathways: cell cycle, apoptosis, and angiogenesis. Most remarkably, we discovered that PAX8 and SOX17 regulate angiogenesis in vitro and in vivo by suppressing SERPINE1, which enables the VEGFR2 signaling pathway. These results have revealed novel therapeutic strategies that could overcome ovarian cancer heterogeneity and resistance, leading to new drug discovery.

3. EGF-mediated signaling impacts nuclear architecture and gene regulation

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Epigenetic modifications regulate gene expression through histone tails. The well characterized Histone modification H3K9me2 is highly associated with gene repression and LADs. However, our data suggest phosphorylation of histone H3K9me2S10 that is triggered by stimulation of cell surface receptors can reverse this gene repression. This is accomplished by inducing DNA dissociation from LADs, which allows pathway specific gene expression to occur. Furthermore, we observed decreased pathway specific genes associating with LADs after EGF stimulation and a corresponding increase in serine 10 phosphorylation. This process allows the cell to rapidly respond to an environmental stimulus through a novel gene expression regulator.

4. *Tropomyosin 1* genetically constrains *in vitro* hematopoiesis

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Increased understanding of genetic mechanisms that regulate hematopoiesis could yield insights into clinical disorders and augment translational efforts to generate blood products *in vitro*. Platelet size and count are heritable and quantifiable outcomes of hematopoiesis. Genome wide association studies (GWAS) have linked hundreds of DNA loci with altered human platelet traits, but little is known about how these GWAS loci mechanistically impact hematopoiesis. Using genome-wide association and epigenetic data sets, we applied a machine-learning framework to identify epigenetic features enriched at established platelet trait association sites. From these results, we derived a quantitative prediction model that identified hematopoiesis-, megakaryocyte- and platelet-relevant genomic loci and related genes more accurately than any prior method. In addition to specifying loci known to regulate hematopoietic traits and functions, our model highlighted novel variants in established platelet trait variation loci. Among nominated loci were variants that alter *Tropomyosin 1* (*TPM1)* gene expression. TPM1 regulates cytoskeletal biology in many cell types and cytoskeletal functions critically impact blood cell biology. With CRISPR/Cas9-mediated genome editing, we created *TPM1*-knockout human induced pluripotent stem cells. *TPM1KO* cells were healthy and showed normal early hematopoietic development. Unexpectedly, *TPM1KO* cells generated twice as many hematopoietic progenitors as controls, increasing total red blood cell and megakaryocyte yields. *TPM1KO* megakaryocytes showed normal morphology, gene expression, and function. These findings help explain human genetics associations and identify a novel strategy to enhance *in vitro* hematopoiesis.

5. The conserved transcription factor *mef-2* regulates sickness induced sleep

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Sleep disruption is a common feature of neurodevelopmental disorders. As such, gaining a detailed mechanistic understanding of sleep circuit development will help elucidate causative factors in neurodevelopmental sleep disorders. Previous work has illuminated aspects of the development of sleep controlling neurons ALA and RIS, but much is yet to be discovered regarding the larger sleep circuit and how sleep signals are interpreted by the body. *C. elegans* experience stress or sickness induced sleep (SIS) in response to infection, radiation treatment, heat shock, or osmotic stress. During SIS, the ALA neuron is activated by Epidermal Growth Factor (EGF) to release the sleep-inducing neuropeptides FLP-13, FLP-24, and NLP-8. Differential effects of these peptides on feeding and movement quiescence suggest that parallel pathways regulate different aspects of quiescence during sleep. Myocyte Enhancer Factor 2 (MEF-2) is a transcription factor that regulates the expression of genes involved in development of both muscles and neurons. In *C. elegans*, *mef-2* regulates muscle to neuron signaling at the neuromuscular junction. We found that animals lacking *mef-2* function are defective in movement, but not feeding quiescence during SIS. *Mef-2* mutants are resistant to EGF, but not FLP-13 overexpression induced movement quiescence. This suggests that *mef-2* functions in the development of the ALA neuron. Surprisingly, we find that muscle (*myo-3* promoter), but not neural (*rab-3* promoter) *mef-2* expression, rescued the SIS defects in these animals. Our working hypothesis is that *mef-2* regulates the development of neuronal sleep circuitry by acting in muscle cells.

6. Contribution of tau, TDP-43, β-amyloid and α-synuclein to medial temporal lobe atrophy in Alzheimer's Disease

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Alzheimer's disease (AD) is defined by the presence of abnormal amyloid and tau proteins. Medial temporal lobe (MTL) atrophy is associated with tau pathology. Pathologies such as TDP-43 or α-synuclein often co-occur in individuals with AD. The aim of this study was to examine the pattern of MTL atrophy associated with different proteinopathies. We also determined the sensitivity of MTL measures to the presence of TDP-43 pathology. Tau, TDP-43, amyloid, and α-synuclein were semi-quantitatively rated in the MTL in 122 individuals with AD pathology. Each individual underwent MRI prior to death. Images were segmented into 6 MTL subregions: anterior/posterior hippocampus (aH and pH), ERC, Brodmann areas (BA) 35 and 36, and PHC. Spearman's correlations were performed with corrections for time between MRI and autopsy, between each proteinopathy, and all MTL subregions. This was followed by partial Spearman's correlations in which each proteinopathy was included in the same model. Receiver Operating Characteristic (ROC) curves were analyzed for each region in order to discriminate TDP-43 negative (n=90) and positive (n=30) patients. Negative associations between tau and pH were found, while TDP-43 was associated with aH and ERC. Using partial Spearman's rank correlations, only TDP-43 was significantly associated with aH and ERC. The aH showed a higher area under the ROC curve (0.8) in order to detect TDP-43 pathology. This study demonstrated specific contributions of different pathologies on MTL substructures. TDP-43 appeared as a relatively stronger modulator of atrophy in the anterior MTL, while tau was strongly associated with the pH. The volume of aH might be a relevant tool to detect the presence of TDP-43. This is crucial, as there is no available biomarker for TDP-43.

7. Dll1^{+} quiescent tumor cells contribute to chemoresistance and metastasis in luminal breast cancer

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Luminal breast cancer accounts for 70% of breast cancer cases. Advanced stage luminal breast cancer patients often show a brief initial beneficial response to chemotherapy, but many of these patients eventually develop resistance to treatment, with subsequent relapse and increased metastasis leading to poor clinical outcome. Using genetic knockout and reporter mouse models, we demonstrate that the Notch ligand Dll1 is important for tumor burden, metastasis, and chemoresistance in luminal breast cancer. We further show that Dll1$^+$ cells are quiescent tumor-initiating cells (TICs) that are responsible for the development of chemoresistance in these tumors. Dll1$^+$ cells avoid chemotherapy by resisting DNA damage and cell death. Pharmacological inhibition of Dll1 completely resensitizes the Dll1$^+$ TICs to chemotherapy. Our data suggest that combining Dll1 antibody with chemotherapy may significantly improve the clinical outcome of luminal breast cancer patients.

8. ABSTRACT WITHDRAWN

9. “CLIP”ping along to a broader understanding of post-transcriptional regulation mediated by Esrps

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The Epithelial Splicing Regulatory Proteins (Esrps), ESRP1 and ESRP2, are highly conserved paralogous proteins required for organogenesis of multiple organ systems and compromised function of Esrps contributes to human diseases and pathologies. Esrps are robustly expressed in the epithelial cells of a variety of tissues, where they are vital in promoting an epithelial splicing network. We perform RNA sequencing on the gastrointestinal epithelium (GI) of mice with conditional tissue specific ablation of *Esrp1*, and global ablation of *Esrp2*. We find that there are largescale changes in alternative splicing and gene expression. We identify 877 splicing events in 720 genes; additionally, 1416 genes were differentially expressed in the GI following ablation of Esrps. To further assess roles of Esrps, we perform enhanced crosslinking immunoprecipitation (eCLIP) to determine ESRP1 protein-RNA interactions in the GI. We observe that ESRP1 not only binds within the introns, but also within the 5' and 3' UTR, of protein coding genes. Notably, 28% of the differentially expressed genes had peaks for ESRP1 in the 3'UTR. Loss of Esrps contribute to significant changes in gene expression and alternative splicing, and ESRP1 appears to directly function in regulating gene expression as determined by its presence in or near spliced introns and exons, and in the 3'UTR of differentially expressed genes. We and others have established a role for Esrps in splicing, and the presence of of CLIP peaks for ESRP1 in 3'UTR suggest novel functions in RNA metabolism. Collectively, my research is elucidating the functions of the Esrps in the epithelial post-transcriptional gene regulatory network.

10. Regulation of breast cancer progression by hypoxic exosomes

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Exosomes (Exo) are extracellular vesicles produced by all types of cells with emerging roles in cell-to-cell communication. Exo production increases in cancer cells in stress conditions, such as hypoxia. Intra-tumoral hypoxia is a common occurrence in advanced breast cancer, correlating with increased risk of metastasis. How hypoxia influences metastatic competence in these settings is not fully understood. We isolated Exo produced by ER+ MCF7 or T47D cells in normoxic (Exo^{NORM}) and hypoxic (Exo^{HYP}) conditions (5% 0_2 or 1% 0_2 for 24 h). To study their biological effect, we used as recipient cells breast epithelial MCF10A, in which we investigated regulation of cell motility, migration and invasion, such as mitochondrial trafficking and dynamics. To elucidate a potential mechanistic basis for this response we screened both transcriptional changes (RNA-Seq) and modulation of kinase activation (Western blotting). We found that Exo^{HYP}, but not Exo^{NORM}, strongly increase the migration and chemotaxis of recipient MCF10A cell line. Biochemically, this was associated with increased phosphorylation of Akt/mTOR kinases. Moreover, Exo^{HYP} promoted increased mitochondrial dynamics, and stimulated the subcellular trafficking of mitochondria to the cortical cytoskeleton of recipient cells, a process that has been linked to increased cell movements. Finally, a genome-wide RNA-Seq screening revealed that Exo^{HYP} induce a strong transcriptional response in recipient cells with activation of gene networks associated with cell motility and migration. Taken together, these data suggest that Exo released by breast cancer cells under hypoxic conditions can reprogram transcriptional and mitochondrial networks of cell motility and invasion in normal epithelial recipient cells.

11. Homology-independent intron targeting enables scalable proteome-wide tagging

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Creating protein fusions by tagging genes endogenously has been revolutionized by the advent of CRISPR-based tools. However, the deliberate and low-throughput nature of current tagging strategies, such as those based on homology-directed repair, has restricted most tagging experiments to a small number of single genes and proteins, as opposed to tagging genes at scale which would enable characterizing the endogenous dynamics of large sets of proteins simultaneously. Homology-independent intron targeting is a scalable, flexible, and efficient gene tagging strategy that utilizes a generic DNA donor. We propose to use this approach to create a pooled proteome-wide tag cell library, where each cell expresses a single fluorescent protein-tagged gene. This targeting-based approach, as opposed to random integration, is important for the creation of a comprehensive proteome-wide tag library and will further allow us to create and optimize a genome-wide map of viable internal protein fusion sites. Furthermore, this library will enable sensitive quantitative proteomics via genetics, as we will be able to sort cells into multiple groups based on fluorescence intensity and identify tagged genes by deep sequencing.

12. Synthesis and *in vitro* testing of a flexible molecular chassis for bacterial infection imaging and transgenic cell monitoring

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The ability to measure and monitor a bacterial infection in human patients with a sensitive and specific imaging agent would be a great advance. Similarly, the ability to track genetically engineered cells *in vivo* would support the development of new cell-based therapies. To this end, we have synthesized a multimodal molecular chassis for imaging agents, TMP-xOTA. This scaffold contains three distinct regions: the protein binding region (TMP); a flexible linker; and the radionuclide binding region (DOTA). Preliminary data in bacteria shows TMP-DOTA has a MIC of 3 μM in *S. aureus,* whereas in *E. coli* the MIC is approximately 30 μM. We began testing of TMP-xOTA tracking of engineered cells in a model system, which shows that unchelated TMP-DOTA is able to enter mammalian cells to bind *e*DHFR with 1 μM affinity *in vitro*. To test for radiochemical uptake, we expressed a stable, codon-optimized version of eDHFR in HEK 293T cells, and TMP-DOTA was radiolabeled using ^{68}Ga with 92% efficiency. Unfortunately, preliminary cell-uptake data suggests no radiochemical accumulation in cells containing eDHFR. There appears to be some non-specific accumulation in WT and TMP blocked cells, which has been shown in the past with fluorescent TMP probes that contain an alkyl chain linker. Second and third generation strategies should facilitate rapid clearance from WT or blocked cells; furthermore, a cell surface expressed eDHFR could offer a better binding target for mammalian cells. In summary, we have synthesized new TMP-xOTA molecules which, in bacteria, have shown a mild selectivity for *S. aureus* over *E. coli*; and in HEK 293T cells, TMP-xOTA can cross the cell membrane and bind the protein of interest, eDHFR.

13. Combination inhibition of WEE1 and ATR causes tumor regression in a CCNE1 dependent manner

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Cyclin E (*CCNE1*) is an oncogenic driver that is amplified in 25% of high grade serous ovarian cancers (HGSOC) and 45-50% of high grade subtypes of endometrial cancer (EMCA). Cyclin E overexpression is associated with poor survival and effective therapies yielding complete and durable responses are lacking. We hypothesized that dual inhibition of WEE1 and ATR (WEE1i-ATRi), which target cell-cycle checkpoints critical for *CCNE1*-amplified cell survival, will result in increased tumor regression with lower dosing strategies. We found that induction of cyclin E upregulates the ATR/CHK1/WEE1 pathway and dual inhibition of WEE1 and ATR resulted in G2/M arrest, mitotic catastrophe, double-strand breaks, apoptosis and synergistically decreased cell viability and colony formation in a *CCNE1* level dependent manner. Combination WEE1i-ATRi treatment was tolerable and resulted in a significant increase in survival in *CCNE1* amplified and high copy gain PDX models compared to WEE1i monotherapy. This combination was less active in a non-amplified/gain, high cyclin E expressing PDX model. Our findings suggest, by preselecting tumors with high *CCNE1* copy number, lower doses of WEE1i-ATRi will be required for tumor killing, which should lead to sparing of normal cells and improved tolerability. Notably, our data indicates that high *CCNE1* copy number may be more predictive of response to WEE1i-ATRi treatment than cyclin E expression alone, indicating that copy number changes in *CCNE1* should be included as a biomarker for the design of clinical trials evaluating WEE1i combinations.

14. 4-1BB agonism overcomes resistance to immune checkpoint inhibitors in treatment-refractory melanoma

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Melanoma is the fifth most common cancer in the United States. Treatment with the immune checkpoint inhibitors anti-CTLA-4 and anti-PD-1 has significantly improved outcomes in patients with metastatic disease; however, more than half of patients treated with one of these agents still fail to achieve durable remission. Studies suggest that combination therapies consisting of two immune checkpoint inhibitors and/or radiation may overcome resistance to monotherapy. 4-1BB (CD137) is a co-stimulatory receptor expressed by T cells, dendritic cells and macrophages. 4-1BB agonists have shown anticancer effectiveness in several murine tumor models and have been modestly successful in early clinical trials. We hypothesize that 4-1BB agonism can augment the anti-tumor effect of PD-1 blockade in treatment-refractory melanoma and lead to improved survival. In this study, melanoma cells derived from B16-F10 tumors, which are resistant to immune checkpoint inhibitors and radiation, were implanted in C57BL6 mice. Mice were treated with anti-PD-1 alone or in combination with a 4-1BB agonist or anti-CTLA-4, and the anti-tumor response was assessed by overall survival and tumor burden. Immune profiling of tumors and spleens from treated mice was carried out by proteomic, transcriptomic and flow cytometry analyses. We found that dual immunotherapy with 4-1BB agonist and anti-PD-1 (P4 therapy) significantly increased overall survival compared to either agent alone or anti-PD-1 plus anti-CTLA-4 combination. Complete remission was achieved in 45.8% of P4-treated mice, compared to 100% mortality in all other treatment arms. Immune profiling showed that the anti-tumor effects of P4 therapy were mediated by both innate and adaptive immune mechanisms, and we identified macrophage and CD8 T cell genes and proteins associated with treatment response. In conclusion, overcoming resistance to immunotherapy by combining anti-PD-1 with a 4-1BB agonist depends on innate and adaptive immune responses. Our analysis revealed potential molecular targets that might be effective amplifiers of the anti-tumor immune response in treatment-refractory melanoma.

15. CELF2 protein regulates signal-induced alternative polyadenylation by competing with core processing machinery

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The 3' untranslated region (3'UTR) of human mRNAs contains sequences that play critical roles in controlling protein expression and function. Importantly, 3'UTRs are not invariant for each gene, but rather are subject to regulation by alternative polyadenylation (APA). In particular, broad regulation of APA has been observed during many transitions in cell state, including T-cell activation. However, the proteins and mechanisms driving APA regulation remain poorly understood. CELF2 is an RNA binding protein that plays a major role in shaping the transcriptome of T cells. Previous transcriptome-wide mapping of CELF2 binding to RNA revealed that CELF2 binding is enriched in 3'UTR of transcripts, including its own 3'UTR. However, the functional impact of this prevalent binding of CELF2 to 3'UTRs is not known. First, using CELF2 knockout/knockdown studies, we found that CELF2 protein is both necessary and sufficient to shift the usage of a proximal to a distal polyadenylation site in its own 3'UTR upon stimulation of T cells. Next, by UV crosslinking and mutagenesis, we showed that this CELF2-dependent shift in APA is due to the competition of CELF2 with the polyadenylation enhancers CFIm25 and CstF64. Further, CELF2 binding overlaps with the APA enhancers transcriptome-wide, and almost half of 3'UTRs that undergo T-cell signaling-induced APA are regulated in a CELF2-dependent manner. Moreover, we found that at least a subset of CELF2-dependent APA events regulate protein expression, including RBFOX2, which has been shown to shape splicing programs in many cell types. These studies thus uncover a new mechanism by which APA is regulated in T-cells and demonstrate a previously unrecognized functional role for CELF2 in shaping global 3'UTR identity.

16. Alcohol metabolism contributes to brain histone acetylation

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Emerging evidence suggests that epigenetic regulation is dependent on metabolic state, implicating specific metabolic factors in neural functions that drive behavior. In neurons, histone acetylation relies on the metabolite acetyl-CoA that is produced from acetate by chromatin-bound acetyl-CoA synthetase 2 (ACSS2). Notably, a major source of acetate is via breakdown of alcohol in the liver, leading to rapidly increasing blood acetate. Neuronal histone acetylation may thus be under the influence of alcohol-derived acetate, with potential effects on alcohol-induced brain gene expression and behavior. Here, using in vivo stable isotope labeling in mouse, we show that alcohol metabolism contributes to rapid histone acetylation in the brain in part by direct deposition of alcohol-derived acetyl groups onto histones in an ACSS2-dependent manner. A similar induction was observed with heavy labeled acetate injection in vivo. In a pregnant mouse, exposure to labeled alcohol resulted in incorporation of labeled acetyl groups into gestating fetal brains. In isolated primary hippocampal neurons ex vivo, extracellular acetate induced learning and memory-related transcriptional programs that were sensitive to ACSS2 inhibition. Notably, we showed that alcohol-related associative learning requires ACSS2 in vivo. These findings support a direct link between alcohol metabolism and gene regulation through ACSS2-dependent histone acetylation in the brain.

17. Inflammatory monocytes restrict dissemination of *Yersinia* by trapping bacteria in intestinal granulomas

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The intestinal immune system performs a critical balancing act by tolerating food molecules, environmental antigens and commensal microbes, while simultaneously mounting robust inflammatory responses to enteric pathogens. Dysregulation of intestinal immunity results in inflammatory bowel disease, celiac disease, or infections by intestinal pathogens and sepsis. One such pathogen is the bacterium *Yersinia pseudotuberculosis* (*Yp*), which colonizes the intestinal mucosa and can disseminate to systemic organs causing a potentially fatal plague-like systemic disease. Early control of bacterial infection at the intestinal mucosa is therefore paramount. This task falls largely to innate immune cells, such as inflammatory monocytes. *Yp* infection of systemic tissues is associated with formation of granulomas, organized immunological structures that form during chronic immune stimulation or failure to clear pathogens. Although originally described by Virchow in 1865, the underlying mechanisms of granuloma formation, as well as how they function to contain pathogens, are not well understood. Here, we report for the first time the formation of intestinal granulomas during enteric *Yp* infection, and identify a requirement for monocytes in their function. Although mice specifically lacking circulating monocytes still formed granulomas in the intestinal tract, they showed increased dissemination of bacteria to the surrounding intestinal mucosa and systemic tissues, and rapid onset of host morbidity. We conclude that inflammatory monocytes are critically required for generation of functional intestinal granulomas in response to oral bacterial infection, and that these granulomas are required to prevent bacterial dissemination into the systemic tissues.

18. Development of PMO-based miRNA site blocking oligos to upregulate Utrophin for Duchenne Muscular Dystrophy therapeutics

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Duchenne muscular dystrophy (DMD) is an X linked muscle wasting disease caused by mutations in the dystrophin (DMD) gene. Utrophin, the autosomal homolog of dystrophin, when overexpressed can compensate for the lack of dystrophin in the *mdx* mouse model of DMD. Hence, utrophin upregulation is considered a promising therapeutic approach for DMD. Utrophin expression in muscle is repressed at the post-transcriptional level; in particular by miRNAs (e.g. let-7c) that target the 3'UTR of utrophin. Previously, we have demonstrated that 2'O-methyl phosphothiorate (2OMePS) platform chemistry-based site blocking oligonucleotides (SBOs) can be used to repress the let-7c:utrophin mRNA interaction and upregulate utrophin *in vitro* and *in vivo*. Additionally, we showed that the upregulation of utrophin achieved using the 2OMePS based let-7c SBOs resulted in functional improvement in DMD mouse model. Here, we have designed and tested pharmacologically superior and more clinically relevant phosphorodiamidate morpholino (PMO) backbone-based let-7c SBOs to repress the let-7c miRNA:utrophin mRNA interaction. Five distinct PMO let-7c SBOs were designed spanning different regions of the let-7c target site in the utrophin 3'UTR. The PMO let7-c SBOs were rank ordered using a reporter mouse muscle cell line in which the luciferase gene is flanked by the utrophin 5' and 3'UTR. The hits were confirmed in the C2C12 mouse muscle cell line for endogenous utrophin expression using western blotting. Treatment of 4 week old *mdx* mice with weekly intravenous injections using let-7c SBO-S5 (the most efficient PMO let-7c SBO) at a dose 60-80mg/kg for 5 weeks demonstrated upregulation of utrophin *in vivo* and amelioration of the dystrophic phenotype. The functional improvement achieved suggests that the PMO let-7c SBO mediated inhibition of the let-7c:utrophin interaction is an encouraging therapeutic strategy for DMD.

19. Harmonization of multi-scanner longitudinal MRI neuroimaging data

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Aggregation of neuroimaging datasets from multiple sites and scanners is becoming increasingly common. While this presents opportunities for increased statistical power, it also presents challenges due to systematic scanner effects. We propose a method for the harmonization of multi-scanner longitudinal MRI data based on ComBat, a method originally developed for genomics and later adapted to cross-sectional MRI data. In simulation studies, we assess the statistical properties of longitudinal ComBat. Using longitudinal cortical thickness data from the Alzheimer's Disease Neuroimaging Initiative (ADNI), we demonstrate the presence of scanner-specific location and scale effects. We compare estimates of the association between the baseline diagnosis group and change in cortical thickness over time using three versions of the ADNI data: (1) raw data, (2) data harmonized using cross-sectional ComBat, and (3) data harmonized using longitudinal ComBat.

20. Chronic short sleep initiates gender-dependent limbic system neurodegeneration

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Chronic short sleep (CSS) is prevalent in society and evidence is accumulating for the role of sleep loss in neurodegeneration, including Alzheimer's disease (AD). Sleep loss can accelerate AD pathology in transgenic animals that overexpress either Aβ42 or tau. However, it is not known whether sleep loss can initiate AD-like pathology in non-transgenic mice. The present study tested the hypothesis that CSS in early adulthood would lead to neurodegeneration in old age that resemble the beginning stages of AD. Male and female C57B6 mice were subjected to 12 weeks of CSS (8 hours lights-on sleep loss for 3 days/week) as young adults. Spatial memory tests were performed at 18 months of age. Subsequently, brain tissue was collected and analyzed for neurodegeneration including hippocampal (HC) and entorhinal cortex (EC) atrophy (stereological Cavalier's volumes) and AD-suggestive immunopathology. Control mice had intact spatial memory, while CSS mice did not. CSS mice showed increased volume of the lateral ventricles (51%) and decreased total HC volume (-5%) as well as specific loss in the CA1 (-5%), CA2 (-19%) and EC (-5%). CSS-exposed males had larger volume changes in lateral ventricles and total HC and CA2 volumes. Female CSS mice had no such volume changes. Overall, CSS mice had increased AT8, a marker of phosphorylated tau, in the HC and EC; increased punctate Aβ42 in the HC and increased CD68, a marker of microglial activation, in the HC and EC. When analyzed separately, female CSS mice had increased AT8 and CD68 in the EC only, whereas male CSS mice had increased AT8 in the EC and HC. These results suggest early life CSS in wild type mice can initiate a neurodegenerative process, which appears more significant in males than in females.

21. Pre-Innervated tissue-engineered muscle for volumetric muscle loss repair

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Volumetric Muscle Loss (VML) is defined as traumatic or surgical loss of skeletal muscle tissue beyond the inherent regenerative capacity of the body, generally leading to a severe functional deficit. Autologous muscle grafts remain the prevalent method of treatment, whereas recent muscle repair techniques using biomaterials and tissue engineering are still at a nascent stage and have multiple challenges to address to ensure functional recovery of the injured muscle. Indeed, appropriate somatomotor innervations remain one of the biggest challenges for both autologous muscle grafts as well as tissue-engineered muscle constructs. We aim to address this challenge by developing pre-innervated tissue-engineered muscle comprised of long aligned networks of spinal motor neurons and skeletal myocytes. Here, we developed methodology to biofabricate long fibrils of pre-innervated tissue-engineered muscle using a co-culture of myocytes and motor neurons on aligned nanofibrous scaffolds. Motor neurons lead to enhanced differentiation and maturation of skeletal myocytes *in vitro*. These pre-innervated tissue-engineered muscle constructs when implanted *in vivo* in a rat VML model significantly increase satellite cell migration, microvessel formation, and neuromuscular junction density in the host muscle near the injury area at an acute time point as compared to non-pre-innervated myocyte constructs and nanofiber scaffolds alone. These pro-regenerative effects can potentially lead to enhanced functional neuromuscular regeneration following VML, thereby improving the levels of functional recovery following these devastating injuries.

22. Novel AKT activator SC79 protects airway epithelial cells against Cadmium-induced lung injury

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Activation of constitutively expressed nitric oxide synthase (NOS) isoforms has beneficial effects via increased ciliary beat frequency and antibacterial nitric oxide production in the airway. It is known that activation of Ca^{2+}/calmodulin can activate NOS. However, other signaling pathways that can activate NOS have not been investigated in airway cells. We hypothesized that activation of the AKT/NOS pathway, which also activates nuclear factor erythroid 2–related factor 2 (Nrf2), may have protective effects during lung injury. We tested the responses of airway cells to SC79, a novel AKT activator. To model epithelial injury, we used cadmium (Cd^{2+}), a component of cigarette smoke that can disrupt epithelial barrier integrity. To test if SC79 up-regulates phosphorylated AKT (pAKT), eNOS (peNOS), and/or Nrf2 levels, A549 human type II alveolar epithelial cells were stimulated with SC79 for 2 hours ± pre-treatment with phosphoinositide-3-kinase (PI3K) inhibitor LY294002. Cell lysates were subjected to western blotting. To determine if SC79 could protect against disruption of tight junction integrity, human bronchial epithelial cells were pretreated with Cd^{2+} for 1 hour and then stimulated with SC79 for 2hrs. Transepithelial electrical resistant (TEER) measurements were taken using an epithelial ohm meter. We observed an approximately 2-fold induction of pAKT, peNOS and Nrf2 with SC79 treatment that lasted >2 hours. This induction was blocked with LY294002. Exposure of cells to Cd^{2+} led to an almost complete reduction in TEER, which was blocked by SC79. Our data suggest that SC79 up-regulates pAKT, peNOS and Nrf2 in lung cells through a mechanism involving PI3K. Furthermore, treatment with SC79 protects the epithelial integrity against Cd^{2+}-induced injury.

23. Reciprocal communication between astrocytes and endothelial cells is required for astrocytic GLT1 expression

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Astrocytes, the most numerous cell type in the central nervous system, perform a variety of tasks from clear and release of neurotransmitters to neurovascular coupling, thanks to their localization that allows them to contact synapses and enwrap the vasculature. Glutamate transporter 1 (GLT1) is mainly expressed in astrocytes and is responsible for up to 90% of the removal of glutamate. In addition, GLT1 is a marker of astrocyte maturation. Although *in vivo* GLT1 accounts for 1% of total brain protein, astrocytes *in vitro* express little or no GLT1. We and others have shown that when astrocytes are cultured in the presence of neurons or endothelia the expression of GLT1 increases. We have shown that a ©-secretase inhibitor blocks the effect of endothelia, suggesting that this effect is mediated by Notch. To further study the role of Notch in the endothelia effect, we use primary cell cultures of rat astrocytes grown in the presence or absence of endothelia cells (bEND.3). We have found that bEND.3 activates Notch/Hes5 in astrocytes. There are four known Notch ligands capable of Notch activation (DLL1, DLL4, Jagged1 and Jagged2). To evaluate which of these Notch ligands contribute to the endothelia-dependent effect, we use recombinant Notch ligands, neutralizing antibodies and lentiviral expression of shRNAs against DLL1 or DLL4. Our results suggest that both DLL1 and 4 are involved in the endothelia effect. We then evaluated the expression of these Notch ligands in bEND.3 cells and found that astrocytes increase its expression in endothelia. Understanding the molecular mechanisms involved in endothelia-astrocyte communication is critical to understanding the biology of this interaction, neurovascular coupling and how this interaction is lost in pathology.

24. The mitochondrial protein CARD19 regulates terminal cell lysis downstream of caspase activation and gasdermin cleavage

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Pyroptosis is a form of regulated cell death during which inflammatory caspases cleave gasdermin D (GSDMD) to release an N-terminal fragment that generates plasma membrane pores, mediating cell lysis and IL-1 cytokine release. However, certain stimuli release IL-1 in a GSDMD-dependent manner while remaining viable in a process termed hyperactivation. How distinct cell fate choices are regulated downstream of caspase and GSDMD activation is unknown. CARD19 is a mitochondrial protein that modulates antiviral responses, but has not been studied during cell death or innate immunity. We identified CARD19 as a potential regulator of a terminal cell death checkpoint downstream of caspase cleavage and hyperactivation during bacterial infection. BMDMs from C57BL/6 and *Card19*$^{-/-}$ mice were infected with *S. typhimurium* or *Y. pseudotuberculosis*. Cell death, protein expression, caspase cleavage, and membrane association were measured by LDH release, western blotting, and membrane fractionation. We found that *Card19*$^{-/-}$ BMDMs were protected from cell death in response to multiple stimuli, but had no discernable defects in caspase activation, IL-1 secretion, or gasdermin cleavage. CARD19 expression was linked to differential cell death in peritoneal macrophages. Mechanistically, *Card19*$^{-/-}$ macrophages exhibited reduced association of cleaved gasdermin D with the plasma membrane. *Card19*$^{-/-}$ mice displayed increased mortality and bacterial burdens following oral bacterial infection. These findings implicate CARD19 as a positive regulator of cell death and a contributor to antibacterial host defenses by facilitating association of cleaved gasdermin proteins with the plasma membrane, in addition to providing insight into terminal cell death regulation following caspase activation and GSDMD cleavage.

25. EWS-FLI1 driven LOXHD1 promotes Ewing Sarcoma metastasis

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Ewing sarcoma is an aggressive malignancy that occurs in adolescents and young adults. Metastasis is the leading cause of death in these patients due to a lack of treatment options. Ewing Sarcoma harbors one of the lowest mutation rates of all cancers, and is driven by the chimeric fusion proteins EWSR1/ETS (EWSR1/FLI1, EWSR1/ERG, etc.), which bind to either a single GGAA motif or at a GGAA microsatellite to deregulate genes involved in cell differentiation, proliferation and migration. However, targeting the EWSR1-FLI1 fusion protein has proven to be unsuccessful in drug discovery. In this study, we developed a bioinformatics pipeline and genetic and epigenetic tools and identified a novel and highly specific EWSR1-FLI1-regulated gene, LOXHD1. Based on cell line studies and two distinct animal models, our data established an oncogenic role of LOXHD1 in promoting EWS metastasis through regulating cytoskeleton homeostasis. Our work highlights that LOXHD1 knockdown in EWS cells may have a synergistic effect with microtubule-targeted chemotherapy medications.

26. Lysosomal de-acidification contributes to antiretroviral-mediated inhibition of oligodendrocyte differentiation

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Antiretroviral therapy (ART) has led to a reduction in the most severe forms of HIV-associated neurocognitive disorders (HAND); however, milder forms of cognitive impairment continue to persist in approximately 30-50% of HIV+ patients. White matter (WM) alterations, including decreased myelin sheath thickness, myelin lesions, and abnormal myelin protein expression, persist despite viral suppression by ART, suggesting that antiretrovirals (ARVs) themselves may contribute to WM structural and functional alterations. While the underlying cellular and molecular mechanisms mediating these effects are currently unknown, work in our laboratory has revealed that ARVs from the reverse transcriptase inhibitor (NRTI), integrase inhibitor (INSTI), and protease inhibitor (PI) classes inhibit oligodendrocyte (OL) differentiation *in vitro* through distinct cellular stress pathways, including oxidative stress and the integrated stress response (ISR). We sought to determine the initiating mechanism upstream of these pathways activated by ART drugs. Using our well-established cell culture system for OL purification and differentiation, we demonstrate that two PIs, darunavir and saquinavir, de-acidify the endolysosome and this contributes to their ability to inhibit OL differentiation. Importantly, this effect was prevented upon co-administration of the TRPML1 agonist, ML-SA1, which re-acidifies the lysosome. Additional experiments utilizing bafilomycin A1 and chloroquine, two drugs known to de-acidify the endolysosome, demonstrate that disruption of lysosomal pH is sufficient to prevent OL differentiation. Future experiments will examine how lysosomal dysfunction initiates downstream cellular stress pathways and may contribute to OL injury in a rodent model of HAND.

27. Evaluating the potential to repurpose statins for ovarian cancer therapy

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Repurposing existing FDA-approved drugs offers an opportunity to accelerate progress in ovarian cancer treatment. Statins are drugs primarily prescribed for hypercholesterolemia and have been scrutinized for decades. Some of this work has demonstrated, through epidemiologic approaches and experimental studies, that statins have anticancer properties. We hypothesized that statins can be repositioned as part of a therapeutic regimen for high-grade serious ovarian carcinoma (HGSOC). Here, we demonstrate that certain ovarian cancer cell lines are exquisitely sensitive to treatment with statins. In addition, we show that the sensitivity to statins is an on-target effect by rescuing with downstream members of the mevalonate pathway. We performed a Reverse Phase Protein Array (RPPA), which revealed that MCL1 levels were altered in statin-sensitive cell lines while there was no such change in non-sensitive lines. After identifying MCL1 as a potential marker of response, Western blot analysis demonstrated a dose-dependent decrease in the protein level of MCL1 with increasing statin concentration. Additionally, a short, pro-apoptotic splice variant of MCL1 was produced upon statin treatment, but only in the sensitive lines. Mechanistically, we find that phospho-YAP (inactive) is upregulated after statin treatment. Using a syngenic cell line system, we were able to demonstrate that the overexpression of YAP increases sensitivity to statins. Interestingly, a constitutively active form of YAP remains insensitive, suggesting the phosphorylation event itself is important. This data corroborates previous research that suggested that multiple different statins decrease nuclear YAP and suggests a mechanism for statin sensitivity. Given these data, the repositioning of statins leads in an exciting new direction for ovarian cancer therapy.

28. Spinal $sPLA_2$ inhibition after nerve root injury prevents pain & modifies glutamate signaling, substance P & cytokines

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Secretory phospholipase A_2 ($sPLA_2$) has been implicated as an early pain mediator in inflammation and neural injury. Although $sPLA_2$ modulates neuronal excitatory signaling and nociception, its effects on pain and spinal responses after nerve root compression (NRC) are unknown. This study investigated whether inhibiting spinal $sPLA_2$ at the time of NRC modulates pain and spinal genes involved in glutamate signaling, nociception, and inflammation early after injury. Male Holtzman rats (n=6/group) underwent a unilateral C7 NRC. Immediately after NRC, the $sPLA_2$ inhibitor thioetheramide-PC was given by lumbar puncture (NRC+TXT). Additional groups underwent either NRC or sham surgery only. Mechanical allodynia was assessed as ipsilateral paw withdrawal threshold at baseline and day 1. On day 1, C6 spinal cord was collected for RT-PCR with primers for glutamate receptors (mGluR5, NR1) and transporters (GLT1, EAAC1), the neuropeptide substance P (SP), pro-inflammatory cytokines (IL1α, IL1β, TNFα), and CyA. Groups were compared by separate ANOVAs with Tukey's HSD. Inhibiting spinal $sPLA_2$ at injury prevents pain onset, with thresholds higher ($p<0.01$) than NRC. mGluR5 mRNA increases ($p<0.03$) in NRC over sham and NRC+TXT; NR1 was not different between groups. GLT1 and EAAC1 sham levels are lower ($p<0.04$) after NRC and NRC+TXT. SP ($p<0.05$) and IL1α ($p<0.02$) NRC levels are elevated ($p<0.05$) over sham and NRC+TXT. IL1β after NRC is higher than NRC+TXT ($p<0.03$) and sham. TNFα is higher after NRC than sham ($p<0.02$) and NRC+TXT. Spinal $sPLA_2$ inhibition prevents early pain onset and upregulation of genes for mGluR5, SP and IL1, which are highly implicated in early pain, suggesting $sPLA_2$ may have a role in early nociceptive mechanisms.

29. Pathogenic variants of *EPHB4* cause lymphatic anomalies through over-activation of mTOR

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Central conducting lymphatic anomaly (CCLA) is a rare and progressive disorder in which lymph is inadequately cleared as a result of dilated lymphatic channels, dysmotility, and/or distal lymphatic obstruction. To understand the genetic causes of the disease, we performed whole exome sequencing on patients diagnosed with lymphatic anomalies. We have identified several pathogenic mutations in *EPHB4* (a gene which encodes ephrin type-B receptor 4 and regulates vascular development), including two missense mutations (c.2288G>A:p.R763Q and c.2654A>G:p.K885R) and a splicing mutation that leads to the use of a cryptic splice donor and the retention of an intervening 12-bp intron sequence (c.2334+1G>C:p.L778_G779insLMLG). Functional characterization using an *in vitro* spheroid-sprouting assay and human lymphatic endothelial cells showed these mutations cause a loss of function of *EPHB4*, and lead to unregulated lymphangiogenic sprouting and development. The functional consequences of these mutations were confirmed in a zebrafish model. Injection of *ephb4a* morpholino into zebrafish larvae resulted in lymphatic vessel misbranching and developmental deformities that mimicked the lymphatic presentation observed in the patients. Further analyses revealed that over-activation of mTOR, resulting from *EPHB4* loss-of-function, led to the observed phenotype. Importantly, the phenotype could be rescued in both the spheroid and zebrafish models upon treatment with rapamycin and the mTOR dual inhibitor OSI-027. These findings demonstrate that these loss-of-function variants of *EPHB4* are implicated in CCLA, and that mTOR inhibitors may have therapeutic benefits in patients with lymphatic anomalies and other vascular disorders resulting from mutations that induce mTOR pathway upregulation.

30. A role for endothelial cell intrinsic IKKα in regulation of immune homeostasis

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Activation of NF-κB in vascular endothelial cells (EC) promotes secretion of inflammatory cytokines and expression of adhesion molecules that contribute to the initiation and maintenance of inflammatory responses. Although the significance of canonical NF-κB signaling in EC has been extensively studied, the role of Non-canonical (NC) NF-κB in these cells is not well understood. To determine the physiological role of NC NF-κB pathway, we developed mouse models targeting IKKα in hemogenic EC by crossing $IKK\alpha^{f/f}$ mice with Tie2-cre animals. The resulting mice lack lymph nodes (LNs) and displayed impaired peripheral B cell maturation. As hematopoietic progenitors are derived from Tie2+ hemogenic endothelial cells, we found that both the EC and hematopoietic compartments lack IKKα in these mice. Despite lacking all peripheral lymph nodes, the spleens were overtly intact in IKKα Tie2 mice. Our experiments characterizing immune cells in these mice resembles the phenotype exhibited by hematopoietic specific IKKα deletion in mice. To determine the EC intrinsic role of IKKα, we crossed $IKK\alpha^{f/f}$ mice with VE-CAD cre and LYVE1 Cre targeting the NC NF-κB pathway in blood or lymphatic endothelial cells (BECs or LECs) respectively. In both these mice, the splenic cellularity and architecture were intact. However, $IKK\alpha^{LYVE1}$ mice lacked all peripheral LNs whereas $IKK\alpha^{VE\text{-}CAD}$ mice had normal peripheral LN numbers but displayed reduced B cell follicles in LNs. We are currently investigating the mechanisms leading to the loss of B cell follicles in these animals. These findings therefore reveal separate functions for IKKα in distinct hemogenic EC-derived compartments regulating immune homeostasis and LN development.

31. Integrative single-cell and bulk RNA-Seq analysis in human retina identified cell type-specific composition and gene expression changes for age-related macular degeneration

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Age-related macular degeneration (AMD) is a leading cause of central vision loss among elderly. Clinical, epidemiologic and pathology studies suggest that AMD preferentially affects distinct cell types and topographic regions in retina. To characterize the impact of AMD on gene expression changes across retinal cell types and regions, we conducted integrative analysis of single-cell RNA-seq data from 92,385 cells generated from 2 donors, and bulk RNA-seq data from another set of 15 donors in macular and peripheral retina. The scRNA-seq data revealed 11 major cell types. Among 75 previously reported AMD risk genes, 29 show cell type- and/or region-specific expression patterns. For example, *CFH* is specifically expressed in endothelium cells, whereas *VTN* and *MMP9* are specifically expressed in photoreceptor cells. To understand the impact of AMD on cell-type composition in retina, we performed cell-type deconvolution analysis in the bulk RNA-seq data using the scRNA-seq data as a reference. Our results highlighted pronounced changes in cell type composition as AMD progresses. Significant changes were loss of rod photoreceptors and increase of microglia and endothelial cells in macula. To investigate the celltype-specific response to AMD, we developed a calibration-based method, identified 1,158 AMD associated genes that are DE only in specific cell types. Among these genes, 126 are specific to rods with 41 up-regulated and 85 down-regulated. Interestingly, the down-regulated genes are enriched in visual perception and detection of light stimulus, whereas those up-regulated genes are enriched for negative regulation of cell death. Taken together, our results reveal changes in cell type composition and gene expression in the macula that are absent in peripheral retina.

32. Polyfunctional CD4+ T cells are efficiently induced by a live influenza virus vaccine but not by inactivated virus

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In the conventional model, CD4+ T cells are activated by antigens presented on MHC class II (MHCII) via an exogenous route, i.e. originating outside the presenting cell. However, our previous work in an influenza virus (flu) system shows that CD4+ T cells are stimulated far more efficiently when MHCII-associated antigen is endogenous, or synthesized within the presenting cell. Here, we sought to test this model by characterizing the CD4+ T cell responses induced by live vs. killed flu vaccines. Mice were immunized with live flu A/Puerto Rico/8/1934 or a large excess dose of inactivated virus. Greater numbers of flu-specific CD4+ T cells were elicited by live vs. killed virus, as shown by peptide/MHCII tetramer binding or production of the cytokines IFNg, TNF, or IL-2. Of particular note, live virus induced a significantly higher frequency of polyfunctional CD4+ T cells, producing two or more cytokines simultaneously. Furthermore, the percentage of flu-specific CD4+ T cells that was polyfunctional was greater for live vs. killed virus, suggesting that CD4+ T cell function, and not just proliferation, was influenced by vaccine format. This effect did not appear to be explained by a difference in total antigen load or adjuvant effects. Studies are ongoing to investigate effects on T follicular helper cell differentiation.

These data are consistent with a model in which endogenous and exogenous MHCII presentation transmit distinct activating signals to CD4+ T cells, perhaps due to differences in peptide/MHCII complex density and kinetics. Endogenous presentation may drive polyfunctional type 1 cytokine responses, which has been associated with enhanced control of chronic infections.

33. Advanced glycation endproducts induce structural changes in bioprosthetic heart valve materials

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At present, the only treatment for severe heart valve disease is either valve repair or replacement with a prosthesis. Bioprosthetic heart valves (BHV) are derived from glutaraldehyde crosslinked heterografts, such as bovine pericardium (BP). Their functional lifespan is limited due to structural valvular degeneration (SVD). Glycation is a process by which glucose or other hexoses are non-enzymatically, post-translation, covalently bound to proteins, resulting in the formation of advanced glycation end products (AGE). AGE can form protein crosslinks, interact with an inflammatory receptor, and modify serum proteins. Immunohistochemistry assays by our group confirmed AGE and serum protein accumulation in BHV clinical explants. Our hypothesis is that AGE formation in BHV results in aberrant collagen remodeling that contributes to SVD. Furthermore, this remodeling is enhanced by serum protein binding.

BP samples (n=5 per group) were studied with incubations for up to 60 days in either: saline (control), 50mM glyoxal, 5% bovine serum albumin (BSA), or 50mM glyoxal + 5% BSA. The substrates were then imaged via secondary harmonic generation (SHG) microscopy. At 24 hours, samples from all incubations had uniformly aligned collagen. At 14 days, only the glyoxal and glyoxal + BSA samples had diminished uniformity in collagen alignment. By 60 days, the control samples demonstrated minimal collagen disorganization. More advanced collagen disorganization was observed in each of the experimental incubations. The most dramatic change was in the co-incubation, glyoxal + BSA where the individual fibrils became unidentifiable. AGE disrupts collagen structure in BHV and this effect is enhanced by BSA.

34. Acute vascular effects of e-cigarette exposure detected by multi-scale MRI and cellular biomarkers

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Electronic cigarette (e-cig) aerosol contains substances potentially deleterious to the vascular endothelium. Here, we tested the hypothesis that inhalation of nicotine-free e-cig aerosol causes acute endothelial dysfunction, and that these effects can be quantified by MRI, along with serum markers of oxidative stress and inflammation. Thirty healthy nonsmokers (mean age ± SD=24.3 ± 4.3years), were subjected to a vaping paradigm using a nicotine-free e-cig. Two blood draws and two MRI examinations (3T, Siemens Prisma) were performed, one each pre- and post-vaping. Specifically, the concentration of nitric oxide metabolites (NOx), resulting from transient changes in nitric oxide, and C-reactive protein (CRP), a marker of inflammation, were assayed. The MRI protocol assessed vascular reactivity to cuff-induced ischemia in the thigh, quantifying luminal flow mediated dilation (FMD) and hyperemic blood flow velocity in the femoral artery (f_a), and hemoglobin O_2 saturation (HbO_2) in the femoral vein. The study group after vaping showed a 34% reduction in FMD, consistent with a lower bioavailability of vasodilatory factors suggested by the decrease in NOx (-20%, from 35.3 to 28.2 μmol/L). Moreover, there was a 2-fold increase in CRP (+95%, from 428.6 to 835.6 ng/ml). These biochemical changes were paralleled by impaired reactive hyperemia in f_a, represented by reduced peak velocity (-17.5%, from 56.6 to 46.7 cm/s) and slope (-26%, from 15.1 to 11.2 cm/s_2), and altered microvascular reactivity, indicated by reduced pre-cuff HbO_2 (-20%, from 65 to 52%). These results suggest that e-cig inhalation elicits transient endothelial dysfunction and activation of an inflammatory response, and that the effect is *unrelated to nicotine*.

35. Loss of both AKT1 and AKT2 in skeletal muscle alters muscle function and induces atrophy via combined activation of FOXO1 and inhibition of mTORC1

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Skeletal muscle (SkM) accounts for more than 40% of the body mass, providing means for locomotion at the expense of cellular energy. SkM relies on distinct metabolic pathways to meet this energic demand. Insulin and insulin-like-growth factor 1 signaling control SkM growth and function and defects in these underlie a host of SkM diseases; however, the molecular mechanisms mediating these effects remain ill-defined.

To understand the signaling mechanisms controlling insulin and IGF-1 regulation of SkM physiology, we generated mice lacking Akt, an essential downstream signaling molecule. Mice were generated with SkM specific deletion of Akt2 (M-Akt2KO), the predominant Akt isoform in SkM; and Akt1 and Akt2 (M-AktDKO), to delete both the Akt isoforms.

Despite a reduction of ~90% of total Akt following Akt2 deletion alone, M-Akt2KO had normal muscle mass and no apparent phenotype. In contrast, M-AktDKO mice have significant reduction in muscle mass resulting in lower absolute force generation and increased fatigability in EDL (glycolytic) and Soleus (oxidative) muscle and impaired performance accompanied by the loss of oxidative fibers. Mechanistically, the reduction in muscle mass in M-AktDKO mice can be rescued *in vivo* by combined activation and inhibition of the mTORC1 and Foxo1 pathways.

36. MYC-mediated transcriptional regulation of mitochondrial chaperone TRAP1 controls primary and metastatic tumor growth

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The role of mitochondria in cancer continues to be debated, and whether exploitation of mitochondrial functions is a general hallmark of malignancy or a tumor- or context-specific response is still unknown. Using different approaches as ChIP seq, RNA seq and CHIP analysis we show that oncogenic MYC proteins, c-MYC and N-MYC transcriptionally control the expression of the mitochondrial chaperone, TNFR-Associated Protein-1 (TRAP1) in cancer. In turn, this preserves the folding and function of oxidative phosphorylation complex II and IV subunits, dampens ROS production and enables oxidative bioenergetics in tumor cells. In addition to bioenergetics, TRAP1 regulation by MYC also shuts down cell proliferation, motility and invasion. All these phenotypes were rescued by re-expressing TRAP1 in MYC deficient cells. Previous studies from our lab have shown that a Hsp90 ATPase inhibitor, Gamitrinib plays an anti-tumorigenic role in prostate cancer. Pharmacologic inhibition of TRAP1 by Gamitrinib, which specifically targets the mitochondrial pool of TRAP1 leads to increase in the cell death of cancer cells. Genetic or pharmacologic targeting of MYC-TRAP1 pathway kills MYC-expressing cells and suppresses primary and metastatic tumor growth, *in vivo*. Therefore, as a target of a ubiquitous MYC oncogene, exploitation of mitochondrial functions is a general trait of cancer, and actionable therapeutic target in the clinic.

37. Can radiation esophagitis in non-small cell lung cancer really be predicted using clinical and dosimetric data?

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Radiation esophagitis (RE) is a common and clinically significant toxicity seen with treatment for locally-advanced non-small cell lung cancer. Prior studies have proposed relevant dosimetric constraints in order to limit this toxicity. We utilize a novel machine learning technique in order to perform additional in-depth analyses of contributing factors to the development of esophagitis in order to uncover previously unidentified criteria, more discrete and robust dosimetric criteria, and the relative importance of individual factors.

Using a machine learning approach to identify predictive features for the development of Grade 3+ RE in a cohort of 203 consecutive stage II-III LA-NSCLC patients treated with definitive chemoradiation at our institution from 2008-2016, we evaluated 32 clinical features per patient grouped into risk factors, comorbidities, pretreatment imaging, stage, histology, radiation treatment, chemotherapy, and dosimetry. Univariate and multivariate analysis were performed using a panel of 11 machine learning algorithms combined with predictive power assessments.

Patients were treated to a median dose of 66.6 Gy at 1.8 Gy per fraction. 11.4% of patients developed grade ≥3 RP. On univariate analysis, no individual feature was found to predict Grade 3+ RE. Multivariate analysis confirmed that no algorithm or feature achieved significant predictive power by AUC threshold.

We find that contemporary machine learning algorithms applied to our modern, institutional patient cohort do not identify any reliable predictors of Grade 3+ RE. This highlights the need to collect and identify novel patient-specific information in order to develop clinically meaningful means to mitigate this survival altering toxicity.

38. Palatable pathways: Nuclear-Localized bitter taste receptors activate calcium signaling, p38 MAP Kinase, and CREB

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Taste family type 2 receptors (T2Rs) are G-protein coupled receptors (GPCRs) involved in bitter taste. T2Rs are also expressed outside the tongue, where they function in diverse roles. In upper-airway epithelia, T2Rs are localized to the plasma membrane and function to detect bitter bacterial quorum-sensing molecules, thereby activating innate immune responses. However, through both immunofluorescence and immunoblotting, we have found that many endogenously expressed T2Rs of the lower-airway epithelia are localized to the nucleus. Nuclear GPCRs are known to be important in some cell types, but nuclear GPCR signaling is not well studied. Many bitter compounds, including bacterial quorum-sensing molecules, are hydrophobic and likely cell permeant; thus, some bitterants may function to activate nuclear-localized T2Rs. Using genetically encoded nuclear-targeted fluorescent indicators, we found that some T2R agonists elevate nuclear calcium and are sensitive to PLC inhibitor U73122, consistent with canonical taste signaling (Gα gustducin and Gβγ activation of phospholipase C). The known calcium-sensitive transcription factor CREB (cAMP response element-binding protein) is activated by phosphorylation from kinases such as protein kinase A or Ca^{2+}/calmodulin-dependent protein kinases. Here, we demonstrate that bitterants induce nuclear calcium release and activate CREB in lower airway epithelial cells.

39. POMC projections from the nucleus tractus solitarius to the mesencephalic trigeminal nucleus control food intake

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The proopiomelanocortin (POMC) system plays an important role in regulating energy homeostasis, in part, through action of the POMC-derived peptide alpha-MSH on the neuroanatomically distributed melanocortin 3/4-receptor (MC3/4R). While food intake control circuits deriving from hypothalamic POMC neurons have been well investigated, the mechanism by which POMC neurons of the nucleus tractus solitarius (NTS) suppress feeding behavior remains unknown. Here, we demonstrate NTS POMC projections to the mesencephalic trigeminal nucleus (MeV), a CNS ganglion involved in the processing of orosensory information and the control of oral motor output. We show, via fluorescent in situ hybridization, expression of MC4Rs on MeV cell bodies. Pharmacological activation of these receptors, via the MC3/4R agonist melanotan-II (MTII), suppresses food intake and body weight. Lastly, the functional relevance of the NTS POMC to MeV projection was analyzed using a Cre-dependent excitatory adeno-associated DREADD virus injected into the NTS of POMC-Cre mice to allow for selective stimulation of NTS POMC neurons with clozapine-N-oxide (CNO). Delivery of CNO directly to the POMC neuron terminals in the MeV recapitulates the food intake and body weight-suppressive effects of systemic CNO delivery. Our work provides evidence that NTS POMC neurons project to the MeV and that MC4Rs in the MeV are sufficient for the suppression of feeding behavior. Future studies are aimed at exploring the role of the MeV MC3/4R in the modulation of satiety via control of oral motor outputs.

40. Quality of life and perceived needs among older adults receiving long-term services and supports

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Long term services and supports (LTSS) are vital for older adults with physical and cognitive disabilities. LTSS can be provided in settings such as nursing homes, assisted living, or via community-based services. The aim of this study is to describe the perceived needs for older adults new to LTSS, examine whether those needs are met in the first three months of LTSS, and determine the relationship with quality of life (QoL). This secondary analysis included data from 470 older adults new to LTSS (average age: 81, 71% female, 51% white, 35% black, 20% Hispanic.) The main outcome of QoL was measured using a single item ("How would you rate your overall quality of life at the present time?"). Perceived needs included supportive equipment devices, transportation, physical therapy, and social activities. Analyses at baseline and three months included t-tests, ANOVAs and simple regression modeling. LTSS recipient reported needs at baseline were: 29% supportive equipment, 31% transportation, 20% physical therapy, and 25% social activities. Those who reported needs at baseline had a lower QoL than those who reported no needs (for all). At three months reported needs decreased by an average of 6% (range: 3%-10%). QoL ratings were associated with changes in physical therapy and social activities needs at three months. The implications of these results related to LTSS recipients' QoL in the first three months of services, with emphasis on physical therapy and social activities needs, is an opportunity to be more person-centered in delivery of care.

41. Structural determinants of Caspase-11 activation by bacterial lipopolysaccharide

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Sepsis is the potentially fatal inflammatory response to bacterial infection. During sepsis caused by Gram negative bacteria, lipopolysaccharide (LPS) is the primary mediator of the inflammatory response. LPS is sensed by macrophages via two receptors: TLR4, which detects extracellular LPS, and caspase-11, which detects intracellular LPS. Activation of caspase-11 takes place when it directly binds to the lipid A moiety of LPS. Lipid A is a conserved phospholipid containing 4-7 acyl chains, depending on bacterial species and growth conditions. Lipid A binding leads to caspase-11 oligomerization, activation, and induction of pyroptosis, a lytic form of cell death that releases inflammatory cytokines, such as IL-1, that contribute to the pathogenesis of sepsis. Previous work has shown that not all LPS structures activate caspase-11 to the same extent. In particular, LPS molecules with fewer than 6 acyl chains have been shown not to activate caspase-11. However, the mechanism by which LPS binding activates caspase-11 remains unknown.

To identify the structural determinants in lipid A that lead to caspase-11 activation, we obtained a panel of lipid A variants that differ in number, length, and position of their acyl chains. We delivered these variants into macrophages and assessed their induction of pyroptosis by measuring cell death and release of IL-1. We have identified for the first time individual acyl chains that determine whether a particular lipid A can induce pyroptosis. We are now using confocal microscopy to visualize the interaction between our lipid A variants and a fluorescently tagged caspase-11 *in vivo*. Understanding the mechanism of caspase-11 activation will be necessary to develop inhibitors that could be used as a treatment for Gram negative sepsis.

42. Mitochondrial targeted biofuels against chemical threats in the multi-model screening platform.

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Chemical agents used in industry, agriculture and as warfare share a common mechanism of toxicity that is mitochondrial dysfunction. To investigate the mitochondrial toxicity of different chemical agents and to evaluate the efficacy of cell-permeable succinate prodrugs, we created a one of a kind screening platform combining human cells, zebrafish and rodents. Human platelets, zebrafish and rats were exposed to moderate doses of the organophosphates chlorpyrifos [CPF] and diisopropyl fluorophosphate [DFP], the carbamate N-methyl N-succinimidyl [NSNM] or sodium fluoroacetate [SF] and subsequently treated with succinate prodrugs (NV). Relevant physiological parameters were evaluated in vivo and mitochondrial function was assessed across all models. Analysis of differences was performed using parametric tests.

The exposure of platelets to CPF, DFP, NSNM and SF inhibited Complex I-linked respiration or upstream metabolism while Complex II was spared. Treatment with NV normalized respiration of CPF, NSNM and SF intoxicated platelets and attenuated the chemically-induced increased lactate production. Zebrafish exposed to CPF and SF presented decreased heart rate and touch response. Rats intoxicated with SF presented with clinical deterioration and inhibition of respiration of isolated heart mitochondria.

In conclusion, cell-permeable succinate prodrugs counteract mitochondrial dysfunction induced by organophosphates, carbamates and sodium fluoroacetate *in vitro*. *In vivo*, sodium fluoroacetate impaired respiration and caused clinical deterioration, which may be rescued with succinate prodrugs. A countermeasure improving the function of a common toxicological target would be a widely applicable treatment against chemical threats.

43. Myasthenogenicity of the syngeneic main immunogenic region in mice

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Myasthenia gravis (MG) is a T cell-dependent antibody-mediated autoimmune disease, characterized by muscle weakness due to impaired neuromuscular transmission. There is an unmet need for developing antigen-specific therapies which are safe and effective. Experimental autoimmune MG (EAMG) is usually induced by immunizing animals with *Torpedo* electric organ acetylcholine receptors (AChRs). In MG and EAMG, more than half of the autoantibodies to muscle AChRs target the main immunogenic region (MIR) of the AChR α1 subunits. A chimera of the human MIR with parts of ACh binding protein (AChBP) induces EAMG in rats[2]. We now develop a syngeneic EAMG model in mice using a new AChBP chimera encompassing the mouse MIR as the immunogen. We will investigate: (1) whether the specificities of the mouse autoantibodies are similar to those found in MG; (2) whether immunized mice develop an autoimmune response to endogenous AChRs; (3) whether, if that is the case, the autoimmune response is self-sustaining. This novel syngeneic model will have implications for the etiology and pathogenesis of human MG, and will provide a new platform to investigate the roles of specific immune components and to test potential new therapies.

44. Surgically induced immunosuppression limits photodynamic therapy efficacy: local to systemic mechanisms

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Lung-sparing radical pleurectomy with intraoperative photodynamic therapy (PDT) promisingly extends survival for patients with malignant pleural mesothelioma (MPM). Nevertheless, most people treated with this approach go on to develop local tumor recurrence, so it is crucial to determine the potential mechanisms that prompt treatment failure and identify strategies to mitigate this. Surgery in the absence of PDT is known to induce inflammation, and we have seen in our pre-clinical models of murine MPM treated with simulated surgery (tumor injury without cytoreduction) followed by Photofrin-PDT that surgery diminishes the curative potential of PDT. To further explore the mechanisms by which surgically induced inflammation might diminish PDT efficacy, we have used these murine MPM tumor injury/PDT models to determine key leukocyte players in the pathway from local tumor response to the establishment of long-term systemic tumor control. Using flow cytometry-based immunophenotyping and functional studies focusing on myeloid-derived suppressor cells and T cells, we have found markedly different patterns of innate and adaptive inflammatory cells in tumors, tumor draining lymph nodes, and spleens of MPM tumor bearing animals. Overall, these studies suggest that surgically-mediated modulation of immune cell trafficking and functionality prior to PDT lead to a systemic suppression of PDT-induced anti-tumor immune response. Targeted inhibition of these molecular or cellular signals of surgically induced inflammation can potentially restore PDT efficacy in the intraoperative setting.

45. Characteristics of diagnosed concussions in children 0-4 years of age

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Children ages 0-4 years have the second-highest rate of emergency department (ED) visits for traumatic brain injury. Despite increased attention, there continues to be a lack of focus on concussion burden in young children. The purpose of this study was to comprehensively describe the natural history and presentation of concussion in children 0 to 4 years of age. To further our understanding of the history and presentation of concussion in young children, a retrospective cohort study of 333 patients aged 0-4 given an ICD-9-CM concussion diagnosis was conducted. The Electronic Health Record was used to capture all concussion clinical care visits. The sample was primarily male (58.1%), had private insurance (53.5%), and were non-Hispanic white (46.5%). The primary mechanism of injury was a fall (64.4%). The time to presentation was commonly the same day of injury (56.2%). More than three-fourths of patients (87%) sought care in the ED or urgent care. Almost half of patients (48%) had only a single clinical episode of care. Most patients or their caregiver (63.1%) reported 1 to 3 distinct Post-Concussion Symptom Scale symptoms. Almost two-thirds of patients (64%) reported at least one somatic symptom, most commonly vomiting, followed by headache and nausea. Additional symptoms included sleep problems (49.2%), emotional changes (21.9%), personality change (34%), change in appetite (12.8%), loss of consciousness (10%) and mental status change (9.7%). These results provide insight into the presentation and clinical characteristics of concussion in young children which will aid clinicians in diagnosing concussions in this age range. These findings suggest the potential need to develop additional tools to adequately assess common symptoms in these young children that may not fall within standard concussion assessment scales.

46. Phenotypic characterization of natural killer cells from early lung cancer patients

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Our current understanding of natural killer (NK) cells as the major effector FcR_{γ}^{+}cells that mediate antibody-triggered tumor cell cytotoxicity in humans is based on studies with blood NK cells. These blood NK cells consist of two subsets defined as immunomodulatory ($CD56^{bright}CD16^{-}$) and cytotoxic ($CD56^{dim}CD16^{+}$) NK cells, the latter predominating. However, there is no consensus on what NK subsets populate human lung tumors and their tumoricidal capacity. The goal of this study wass to phenotypically and functionally characterize the NK subsets from blood and tumors of early-stage lung cancer patients. Our phenotypic analysis demonstrated that lung tumors predominantly accumulate $CD56^{+}CD16^{-}$NK subset. These $CD56^{+}CD16^{-}$NK cells exhibit a phenotype of tissue-resident NK cells ($CD69^{+}CXCR3^{+}CD62L^{-}CCR7^{-}CD56^{+}CD16^{-}$) that is different from blood $CD56^{+}CD16^{-}$ NK cells. In addition, tumor-resident $CD56^{+}CD16^{-}$ NK cells are characterized by the downregulation of NK-activating receptor CD226 and NK-inhibitory receptor KIR2DL3, and upregulation of the NK-inhibitory receptor NKG2A. Functionally, in contrast to blood NK cells, we found that total tumor-infiltrating NK cells had impaired ability to mediate natural and cetuximab-triggered killing of tumor cell lines due to three interconnected mechanisms. First, we found that tissue-resident NK subset is unable to mediate natural and cetuximab-triggered tumoricidal activity towards tumor cells. Second, the recruitment of the $CD56^{+}CD16^{-}CXCR3^{hi}$NK cells is increased compared to $CD56^{+}CD16^{+}$NK cells from blood. Third, the $CD56^{+}CD16^{-}$ tumor-resident NK cells could represent the population of exhausted cells due to successive rounds of stimulation by primary tumor cells. Interestingly, we found that subset of primarily cytotoxic $CD56^{+}CD16^{+}$NK cells in tumor still possess the tumoricidal potential, although at low levels compared to blood counterpart. Thus, the impaired tumoricidal activity of bulk NK cells in tumor could be explained by preponderant accumulation of tissue-resident NK with intrinsically low ability to kill tumors.

47. Unique cell states in the rare genetic female disease pulmonary lymphangioleiomyomatosis (LAM)

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LAM is a rare genetic lung disease that affects women of childbearing age. It is characterized by a metastasizing growth of abnormal smooth muscle-like cells originating from an unknown source, which destroys the lung parenchyma leading to pulmonary failure. Attempts to establish unique cell surface molecular markers of 'LAM' cells and LAM cells origins have not been successful. It is generally known that LAM lesions stain positive for phospho-ribosomal protein S6 (a marker of mTORC1 activation) and smooth muscle α-actin. In a search for the LAM cell origin, we characterized the unique composition of a LAM lung compared to age- and sex-matched control using single-cell transcriptomics. As a result, we identified the following cellular sub-types specific to the LAM lung: (i) transitional state alveolar epithelial cells expressing both alveolar type 2 (AT2) and alveolar type 1 (AT1) cell markers, (ii) "fibrotic" mesenchymal alveolar niche cells (MANCs), characterized by the gene signature of canonical MANCs (PDGFR□, DCN, SFRP4, WIF1), but additionally expressing pro-fibrotic gene markers CTGF, COL1A1 and ACTA2, (iii) fully differentiated smooth-muscle cell (SMC) subtype 'enhanced' with SMC gene markers ACTA2, MYLK, MYL9, TAGLN, DES, CTSK, and (iv) an undefined cell type, exhibiting expression of both endothelial (VCAM1) and epithelial (KRT18, MSLN) gene markers. The latter cell type was additionally characterized by the expression of known LAM-specific genes VEGFD, PLAUR, CCL2, and MYH10. Additionally, we found that the main source of signaling in the LAM lung was the mitogen-producing MANCs. Notably, the cross-talk was enriched specifically between MANCs and the newly observed LAM-specific cell sub-types, indicating that MANCs may be responsible for their formation.

48. Mapping the facultative gene regulatory landscape of lung alveolar epithelial progenitors

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Typically, lung alveolar tissue is quiescent with minimal cellular turnover. However, epithelial alveolar type 2 cells (AT2) maintain a facultative regenerative capacity. A subset of AT2 cells termed alveolar epithelial progenitors (AEPs) are Wnt responsive, express the Wnt target gene Axin2, and contribute to robust alveolar regeneration. The precise role of Wnt signaling in alveolar regeneration remains unclear, although our recent work has suggested it balances the self-renewal of AEPs with differentiation into AT2 and alveolar type 1 (AT1) cells. Moreover, the extent to which AEPs are pre-defined during development and maintained during adulthood is ambiguous. If subsets of AT2 cells such as AEPs are maintained with unique regenerative properties, then these cells should be defined by distinct gene regulatory states. Ostensibly, given the facultative nature of AEPs, these gene regulatory states should be developmentally defined and maintained throughout maturation.Integrating available RNA-seq, ATAC-seq and whole lung scRNA-seq data, we have identified a putative set of transcription factors specific to AEPs including the grainyhead/CP2 family transcription factor Tfcp2l1. Tfcp2l1 was previously shown to be a Wnt responsive gene and thus could mark the AEP sublineage in a fashion similar to Axin2. Importantly, Tfcp2l1 is known to repress lineage commitment in mouse ES cells suggesting that it could play a functional role in maintaining the multipotent state of AEPs. Future directions include determination of the lineage commitment of Tfcp2l1 expressing cells after acute lung injury, as well as testing the requirement for Tfcp2l1 gene expression in AEP regenerative ability.

49. CDK7 inhibition suppresses castration-resistant prostate cancer through MED1 inactivation

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Metastatic castration-resistant prostate cancer (CRPC) is an aggressive disease with high mortality rate resulting primarily from transcriptional addiction driven by androgen receptor (AR). First-line CRPC treatments typically target AR-signaling, but are rapidly bypassed, resulting in only a modest survival benefit of the anti-androgen therapies. Therefore, molecular approaches that more effectively block the AR-transcriptional axis are urgently needed. Here, we investigated the molecular mechanism underlying the association between the transcriptional co-activator MED1 and AR as a vulnerability in AR-driven CRPC. MED1 undergoes CDK7 dependent phosphorylation at T1457 and physically engages the AR at super-enhancer sites, and is an essential determinant for AR-mediated transcription. Additionally, a CDK7 specific inhibitor THZ1 blunts AR-dependent neoplastic growth by blocking AR/MED1 co-recruitment at a genome-wide level, as well as results in reversion of the hyper-phosphorylated MED1 associated enzalutamide resistant phenotype. *In vivo*, THZ1 induces tumor regression of AR amplified castration-resistant human prostate cancer in xenograft mouse model. Together, these results demonstrate that CDK7 inhibition selectively targets MED1-mediated, AR-dependent oncogenic transcriptional amplification, thus representing a potential new approach for the treatment of advanced prostate cancer.

50. Identification of effectors of TNFα signaling that modulate liver repopulation by high throughput *in vivo* CRISPRa screening

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Overexpression of TNF receptor 1 (TNFR1) in hepatocytes significantly inhibits liver repopulation following toxic liver injury. However, the cascade downstream of TNFR1 activates both pro- and anti-apoptotic signaling. Using CRISPR/Cas9 gene activation (CRISPRa) to perform a screen in the *Fah*$^{-/-}$ model of toxic liver injury and repopulation, we tested two hypotheses: (1) Specific effectors downstream of TNFR1 promote liver repopulation and (2) Different transcriptional activator (TA) components of CRISPRa will result in varying levels of gene activation and toxicity *in vivo*. *Fah*$^{-/-}$ mice maintain normal liver function when continuously treated with the drug nitisinone. Expression of a functional copy of *Fah* in hepatocytes of *Fah*$^{-/-}$ mice, followed by withdrawal of nitisinone stimulates the formation of nodules of rescued tissue. To screen for promoters of liver repopulation, we hydrodynamically injected *Fah*$^{-/-}$*dCas9*$^{+}$ with transposon/transposase plasmid libraries encoding a functional *Fah* cassette, one of four different TAs, and 1250 guide RNAs (gRNAs) targeting 119 downstream effectors of TNF signaling, along with 100 non-targeting control gRNAs. After liver repopulation, gRNAs targeting the promoter of *Birc2,* an inhibitor of apoptosis, were enriched in mice injected with three of the four CRISPRa systems, while gRNAs targeting *Casp8*, an initiator of apoptosis, were depleted in mice injected with two of the four models. By performing these gRNA screens, we have identified key components of the TNF pathway, namely regulators of apoptosis, as key factors involved in the regenerative response to toxic liver injury. In addition, we have optimized CRISPRa and shown that it is a viable method to evaluate entire genetic pathways in the hepatocytes repopulating the liver.

51. Harnessing the dynamic microtubule end: lessons from in vitro reconstruction studies

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Dynamic microtubule end is a highly complex assembly of tubulin dimers with different conformations and biochemical composition. Various microtubule-associated proteins can bind to and modify these variable structures, regulating dynamics of the microtubule ends and attaching them to specialized cellular sites, such as the kinetochores. To learn about how microtubule ends behave when bound to kinetochores, we reconstruct these interactions using purified tubulin and microtubule-binding proteins. A promising novel approach to reconstruct the microtubule end-attachment sites is to employ DNA origami scaffolds, which can be programed to have precisely controlled stoichiometry and spatial positioning of conjugated proteins. We synthesized a ring-shaped DNA origami with the outer diameter ~60 nm, which roughly corresponds to the area for a microtubule end binding at the kinetochore. These scaffolds are fluorescent labeled and immobilized on coverslip surface. Using SNAP proteins fused to GFP-binding nanobodies, we conjugated 10-20 purified GFP-tagged microtubule-binding proteins to each scaffold which form clusters. Clusters of Ndc80 complexes, the major microtubule-binding kinetochore component, support lateral diffusion of stabilized microtubules and show weak binding to the microtubule ends, as expected for this microtubule wall-binding protein. However, clusters of the TOG-domain protein CLASP2, which is required for tubulin incorporation at the kinetochore-bound microtubule ends, exhibit strong binding specifically to the plus-ends of stabilized microtubules. We show that CLASP2 recognizes the nucleotide-dependent conformation of the terminal tubulin dimers, providing a novel molecular mechanism to suppress microtubule catastrophe at mitotic kinetochores.

52. High throughput screening of FDA approved drugs with EZH2 inhibitor in prostate cancer cells

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Epigenetics provides promising new targets for anticancer therapy. One such important target is EZH2, which is the enzymatic component of histone methyltransferase PRC2 complex. EZH2 silences gene expression through generation of the lysine 27 trimethylation mark on histone H3 (H3K27Me3) by its catalytic SET domain. Elevated expression of EZH2 is observed in aggressive forms of prostate and breast cancer, as well as multiple other solid tumors and a significant percentage of DLBCL and some rare forms of sarcomas carry gain-of-function EZH2 mutation. Highly specific small molecule EZH2 inhibitors have been developed and have entered clinical trials for any solid cancer with an EZH2 GOF mutation. However, a rationale combination of an EZH2 inhibitor with other targeted therapies will increase the reach of this promising therapeutics in cancers that display high expression of EZH2. Compared to Androgen Receptor (AR) positive metastatic prostate adenocarcinoma, AR-negative neuroendocrine prostate cancer is characterized by extremely high levels of EZH2. Currently, there are no targeted therapies available for neuroendocrine prostate cancer, and therefore EZH2 inhibitor could be a potential therapeutics for this deadly disease. However, in our preliminary data treatment of EZH2 overexpressing prostate cancer cells, LNCaP and DU145 with EZH2 inhibitor (GSK126) did not yield any significant growth inhibition even after 12 days of treatment although histone H3K27me3 mark got completely erased by 3-4 days of drug treatment. We hypothesize that loss of H3K27me3 and associated transcriptional output in these prostate cancer cells by EZH2 inhibitor will create novel synthetic lethal interactions, and screening of small molecule inhibitor libraries will yield novel combinations of drugs that will be synergistic with EZH2 inhibitor in treating neuroendocrine prostate cancer.

53. How to look human: a genetic basis for the evolution of increased sweating capabilities in humans

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In humans, heat dissipation is mainly achieved by the evaporation of sweat from the skin. Key to the effectiveness of this mechanism is the human-specific evolution of a dramatic increase in eccrine sweat glands (ESGs) density. The genetic changes underlying the evolution of this adaptive trait are unknown. In humans, expression of the Engrailed-1 (En1) transcription factor correlates with the onset of ESGs formation. In mice, we previously showed that regulation of En1 expression is a major determinant of natural variation in ESGs density between strains. Increased En1 expression in the epidermis promotes the specification of more ESGs. In light of these findings, we hypothesized that modulation of En1 expression could be an underlying mechanism for the human ESGs density.

The En1 coding sequence is conserved among primates and, in general, many evolutionarily-relevant changes occur in regulatory regions. Therefore, to test the hypothesis that changes in En1 expression impacted human ESGs density, we investigated the regulation of En1 expression in humans and other species. Using comparative genomics combined with functional validation of enhancer activity in mice, we identified a suite of En1 enhancers active during ectodermal appendage development. We then compared the relative activity of the mouse, human, and non-human primate sequences of these elements in human keratinocytes and in mouse developing skin. We find that among these elements, the activity of one enhancer–previously identified as a region of positive evolution in humans–is strikingly higher than that of other primates. Our data indicate that the rapid evolution of an En1 enhancer led to increased En1 expression on the human lineage and contributed to the enlarged ESGs density in our species.

54. Defining the role of DLST in MYCN-driven neuroblastoma

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Neuroblastoma is the most common extracranial tumor in children and is responsible for ~15% of childhood cancer-related mortalities. Aberrant *MYCN* activity is found in ~30% of neuroblastoma cases and is associated with highly aggressive tumors and a poor prognosis. Metabolic reprogramming is a hallmark of cancer and represents a fundamental difference between cancer and normal cells in terms of how they utilize nutrients for energy production and macromolecule synthesis. DLST is the E2 component of the enzymatic complex α-ketoglutarate dehydrogenase complex (KGDHC), which catalyzes an irreversible step in the TCA cycle. We found that elevated expression of *DLST* predicts poor overall survival in human neuroblastoma patients ($p<0.0001$) and tumor aggression ($p<0.001$). Utilizing a zebrafish model of MYCN-driven neuroblastoma, we have demonstrated that heterozygous loss of *dlst* significantly delays tumor onset ($p<0.005$). Additionally, we have shown that *dlst* overexpression in MYCN-driven zebrafish model leads to increased tumor burden and metastasis. To determine the sensitivity of human neuroblastoma cells to DLST inhibition, we genetically inactivated DLST in a panel of neuroblastoma cell lines and found that *DLST* inactivation results in decreased cell viability and increased cell death in all MYCN-amplified cell lines. Taken together, our studies identified DLST as an important mediator of high-risk neuroblastoma with *MYCN* amplification and demonstrated that *DLST* inactivation can kill these cancer cells, which provides compelling evidence that the metabolic dependence of neuroblastoma cells on DLST.

55. A Novel Mouse Tubb4aD249N/D249N to model H-ABC and development of potential Therapeutic strategies

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Hypomyelination and Atrophy of Basal ganglia and Cerebellum (H-ABC) is a rare leukodystrophy with mutation in tubulin alpha 4 (*TUBB4A*) and p.Asp249Asn (D249N) is a recurring heterozygous mutation found in majority of affected individuals. H-ABC begins in infancy, characterized by dystonia, ataxia, altered gait and progressive motor dysfunction. To establish therapeutic strategies, our group developed a knock-in mouse model harboring heterozygous (*Tubb4a*^{D249N}) or homozygous (*Tubb4a*$^{D249N/D249N}$) *Tubb4a* mutations using CRISPR-Cas9. This H-ABC model (*Tubb4a*$^{D249N/D249N}$) displays progressive motor dysfunction with tremor, abnormal gait, ataxia and decreased survival. Further, *Tubb4a*$^{D249N/D249N}$ mice shows delay of myelination and ultimate demyelination with reduced number of oligodendrocytes (myelinating cells in CNS), severe cerebellar atrophy and significant striatal neuronal loss. Thus, *Tubb4a*$^{D249N/D249N}$ mouse recapitulates H-ABC disease, making it a unique model to develop pre-clinical strategy. One approach to treat H-ABC is to reduce *Tubb4a* expression as *Tubb4a Knockout* mouse are developmentally normal, suggesting that TUBB4A is redundant in a cell. To reduce TUBB4A expression in mouse, ongoing studies are focused on screening of shRNA–adeno-associated virus (shRNA-AAV) *in vitro* to establish most effective shRNA-AAV. Subsequently, the best candidate shRNA-AAV will be administered in *Tubb4a*$^{D249N/D249N}$ mouse at different time points and different routes to test if AAV-shRNA treatment can rescue hypomyelination, cerebellar ataxia and tremors. Additionally, dose escalation experiments will be performed to establish dose response curve in mice. This work will serve as pre-clinical proof of principal for testing if use of AAV-shRNA is a viable approach in treatment of H-ABC.

56. Role of amino acid sequence differences in the global intracellular functions of β- and γ- actin

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Actin is one of the most essential eukaryotic proteins, highly conserved across the tree of life. Among the 6 mammalian actins, only β and γ cytoplasmic actins are ubiquitously expressed, and share the highest identity at the amino acid level: only 4 substitutions within their N-termini. The mechanisms maintaining their functional differences *in vivo* are one of the major unresolved questions in the field. We used a mouse model developed in our lab, where β-actin gene is altered to encode γ-actin protein from the endogenous *Actb* locus (*Actbc-g* mice). These mice lack β-actin protein, while the *Actb* gene is nearly intact. While these mice are viable and appear phenotypically normal at the gross level, lack of β-actin protein leads to specific defects in tissues depending on microvilli for their function, including the retina, and the brush border of the small intestine. Both tissues show major morphological changes in *Actbc-g* mice, including damaged microvilli of the retinal pigment epithelium responsible for photoreceptor homeostasis, as well as disorganization of the terminal web and altered microvillus length in the small intestine. These defects in retina are accompanied by progressive loss of light sensitivity. We link these defects to the altered binding specificities of actin associated proteins with β- and γ- actin. Our results suggest β-actin protein is essential for microvilli maintenance in multiple cell types and constitute the first demonstration of contributions from actin isoform's amino acid sequence to actin's *in vivo* functions. This study sheds major light on the functional differences between the two closely related cytoplasmic actins, which universally affect a specific subset of actin functions in multiple cell types.

57. Autophagy is required for the beneficial effects of calorie restriction during irradiation-induced intestinal injury

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Defective autophagy is associated with compromised intestinal integrity and chronic illness, including inflammatory bowel disease. One mechanism of inducing autophagy in other tissues is reducing calorie intake while maintaining nutrients, which can be beneficial in regeneration. However, the relative contribution of autophagy in intestinal regeneration following CR is not known. Our hypothesis is that intestinal epithelial cell-specific autophagy is required for the regenerative benefits of calorie restriction. In this study, wild-type mice or mice with intestinal epithelial-specific deletion of key autophagy gene *Atg7* were placed on either a calorie restricted (CR) or nutrient-matched *ad libitum* diet for 5 weeks. Half of the mice were then subjected to whole body irradiation (12Gy) and allowed to recover for 3 days. First, mice with autophagy deficiency had drastically different weight changes over the 5 week period. Relatedly, following irradiation, wild-type mice lose roughly 15% of their body weight compared to unirradiated controls. In line with previous findings, CR mice had less weight loss than the *ad libitum* group, confirming a cytoprotective effect of CR. CR mice also had increased regenerative foci. *Atg7* deletion mitigated this. CR mice with *Atg7* deletion had decreased regenerative foci compared to wild-type CR mice. Overall, these data support the hypothesis that autophagy is necessary for the regenerative benefits of CR. Clinically, understanding the mechanisms underlying the regenerative benefits of CR will be useful in developing small molecule drugs or other therapeutic strategies that could be used in place of complex, time-consuming dietary regimens.

58. Sorafenib and LXR agonist combination treatment targets hepatocellular carcinoma through metabolic stress

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Hepatocellular carcinoma (HCC) treatments have low efficacy, as FDA-approved drugs including sorafenib extend patient survival by three months. We sought to identify novel druggable targets for use in combination with sorafenib to increase its efficacy. We implemented a genetic screening paradigm using an overexpression library of 43 genes. We administered this library in the context of liver injury and treated mice with vehicle or sorafenib and determined the genetic drivers of each tumor. Mice injected with the screening library developed HCC clones containing MYC cDNA plus various other cDNAs. Treatment with sorafenib resulted in sorafenib-resistant HCCs that were significantly depleted in NR1H3 cDNA, encoding Liver X receptor (LXR), suggesting that LXR activation is incompatible with tumor growth during sorafenib treatment *in vivo.* Combination treatment using sorafenib and LXR agonist GW3965 in multiple HCC cell lines and a primary cell line led to enhanced cell death as compared to monotherapy, due to reduced expression levels of cell cycle regulators and increased expression of genes linked to apoptosis. Pathway analysis revealed alterations in metabolic pathways, and we observed upregulation of gluconeogenic regulators and downregulation of glycolytic regulators in sorafenib and combination treatment, and increased regulation of fatty acid synthesis genes in GW3965 and combination treatment. When gluconeogenesis and fatty acid synthesis are reduced using siRNA, cells are resistant to combination therapy. We propose that sorafenib upregulates glucose synthesis and downregulates glycolysis, while GW3965 upregulates fatty acid synthesis. In combination, sorafenib and GW3965 deplete cellular resources required for ATP production, subsequently leading to apoptosis.

59. Role of Type III Collagen in the Breast Cancer Microenvironment

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Collagen deposition and remodeling play key roles in wound healing, fibrosis, and cancer. Our lab has identified that type III collagen (Col3) promotes a regenerative wound healing response and suppresses aggressive breast cancer behavior in triple negative breast cancer (TNBC) models. Our data reveals that loss of Col3 promotes a profibrotic response, characterized by an increase in myofibroblast density and desmoplasia. Here, we show that human fibroblasts treated with siCol3 produce highly aligned fibrillar collagen matrix (compared to siCtrl). Moreover, culturing TNBC cells on Col3-deficient matrices increased proliferation and decreased apoptosis. Notably, we show that invasive regions of human TNBC samples contain less Col3 and straighter fibers than non-invasive regions. Consistent with the roles of Col3 in wound healing and the tumor microenvironment (TME), incidence and volume of post-resection local recurrence of 4T1 tumors in $Col3^{+/-}$ mice were greater than that of WT mice. Next, Col3 was included during TNBC cell injection in murine models to assess its ability to prevent formation of a permissive-TME. Increasing Col3 in the early TME attenuated growth of primary tumors and suppressed formation of an aggressive TME, characterized by dramatic alterations in collagen organization and increased collagen deposition, gelatinase activity and nuclear YAP staining. Furthermore, the ability of Col3 to increase TNBC apoptosis compared to Col1 support development of Col3 biomaterials for use in post-resection sites of breast cancer patients. These data suggest that Col3 plays a tumor suppressive role in breast cancer and that Col3-biomaterials may provide a safe and effective strategy to improve both post-operative healing and limit recurrence in breast cancer patients.

60. Single cell transcriptomics identifies a unique adipocyte population that regulates bone marrow environment

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The true identity of bone marrow (BM) mesenchymal stem cells (MSCs) and their in vivo bifurcated differentiation routes into osteoblasts and adipocytes remain poorly understood. In this study, we applied single cell RNA-sequencing (scRNA-seq) on Td^+ cells from BM of 1-mo-old *Col2-Cre Tomato (Col2/Td)* mice, whose Td signal labels the entire mesenchymal lineage cells. Unsupervised clustering of 7585 mesenchymal lineage cells yielded 10 clusters. MSC cluster expressed stem cell markers (Sca1 and CD34). Cell trajectory analysis confirmed that as the ancestor cells, MSCs undergo bi-differentiation into osteogenic and adipogenic lineage cells (Fig. 1B). Using a mature adipocyte-specific reporter, we validated the existence of an abundant adipocyte population in 1-mo-old *Adipoq-Cre Td (Adipoq/Td*) mice. Those adipocytes expressed most adipocyte markers, had no proliferative ability, and contained no lipid droplets. In bone, they existed as marrow stromal cells and pericytes surrounding sinusoids. Cell ablation in 1-mo-old *Adipoq-Cre DTR Td* (*Adipoq/DTR/Td*) mice after 2 wk of DT injections resulted in marrow vasculature collapse, as well as de novo trabecular bone formation. Large scale scRNA-seq analysis of Td^+ cells from *Col2/Td* mice received focal radiation (5 Gy) generated two new adipogenic differentiation routes for MBPs. One of them was made of highly proliferative cells with elevated DNA repair ability. Analyzing *Adipoq/DTR/Td* mice confirmed that those cells are essential for rapidly generating enough adipocytes acting as pericytes to repair damaged vessel structure after radiation. In summary, we discovered a novel type of adipocytes that regulate marrow vessels, bone formation, injury repair and named them as marrow environment regulating adipocytes (MERAs).

61. Serotonin Related Gene Expression in Heart Valve Interstitial Cells are Altered by Proliferation and Serotonin Transporter Inhibition

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Serotonin (5HT) has been observed to be associated with valvulopathies with 5HT secreting carcinoid tumors and following administration with 5HT–pharmaceuticals, such as Fenfluramine. Relatively, little is known about anti–depressant drugs such as Fluoxetine (Flx) in terms of their potential for causing valvulopathies. Flx is a selective serotonin (5HT) reuptake inhibitor (SSRI) that prevents binding of 5HT to its transporter (SERT), consequently enhancing the signaling downstream of SERT and 5HT receptors. Our hypothesis is that 5HT related gene expression patterns in aortic and mitral valve interstitial cells (A- and M-VIC) is affected by proliferation and dysregulated by Flx. Pig valve leaflets were dissected from hearts obtained from an abattoir. A- and M-VIC were harvested, from left and right coronary valve cusps, using collagenase, type II. At passage one to two, 80% confluent, cells were trypsinized, and divided into two groups. The control/non–treated group, and experimental group (treated with 1μM Flx), at a final seeding density 1.5×10^5 ml^{-1}. At predetermined time–points, cells were lysed and RNA was extracted for qRT–PCR. A- and M-VIC grown under full serum conditions (10% fetal bovine serum) showed robust growth with nodule formation with or without Flx, by day 7 in all cultures. qRT–PCR results at 2 days and 7 days from A- and M-VIC grown without Flx demonstrated significant upregulation of *HTR2A* and *COL1A2*, and down regulation of *SERT*, *VMAT2* and *HTR2B*. Flx treated A- and M-VIC demonstrated the same trends, but with dysregulation of all genes. In conclusion, 5HT related gene expression changes in proliferating A- and M-VIC are consistent with enhanced *HTR2A* signaling that is known to be associated with *TGF–beta–1* related extracellular matrix production. Flx diminishes the extent of this change in gene expression patterns.

62. The effect of histone post-translational modification on transcriptional bursting during development

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In all eukaryotes studied, transcription at individual gene loci exhibits random oscillations between active and inactive states, a phenomenon known as transcription bursting. This fundamentally impacts the synchrony and robustness of cell fate specification during embryonic development. To understand how transcription controls development, it is necessary to uncover the molecular determinants that control the duration of bursts and the rates at which bursts occur. Eukaryotic transcription is characterized by the enrichment of histone post-translational modifications (HPTMs) at enhancers and promoters. Despite the strong correlation between HPTMs and gene activity, it is unclear how HPTMs determine transcription rates and set bursting frequencies. Here, I have knocked down an array of histone methyltransferases, acetyltransferases, demethylases, and deacetylates during Drosophila oocyte development and determined their effect on the transcriptional bursting. Because these genes are nearly all essential and lethal when lost, I knocked them down maternally and assessed their effects by looking at the transcriptional bursting of the maternally expressed gap gene hunchback. For each maternal RNAi knockdown, I measured the transcriptional dynamics hunchback using single mRNA FISH. By fitting this single mRNA FISH data with a “two-state” mathematical model of transcription, I determined whether each HPTM affected burst duration, frequency, or initiation rate. This screen shows how decreasing or increasing specific histone marks change bursting kinetics and promoter state dynamics.

63. Electrophysiologic and behavioral analysis of alterations in the vHPC-mPFC circuit following traumatic brain injury

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Traumatic brain injury (TBI) affects more than 2.5 million people in the United States each year and is associated with long-term cognitive impairments. Strong unidirectional, monosynaptic glutamatergic projections from the ventral hippocampus (vHPC) to all cortical layers of the medial prefrontal cortex (mPFC) are implicated in various cognitive functions, including spatial working memory. We have demonstrated that TBI leads to diminished cortical layer V field potentials evoked by layer II/III stimulation. This study examined the behavioral and electrophysiological correlates of vHPC-mPFC circuit dysfunction after mild TBI using the lateral fluid percussion injury (LFPI) model of brain injury. Under anesthesia, 6-8 week-old male C57/BL6 mice underwent mild LFPI and behavioral experiments commenced the next day. Performance of sham and LFPI mice in a working memory task, T-maze, was compared up to 7 days after injury. Electrophysiological experiments were performed 6 to 8 days after injury with brain slices cut to isolate vHPC-mPFC projections according to published methods. Extracellular recordings of field excitatory post-synaptic potentials (fEPSPs) were obtained by placing a stimulating electrode in layer II/III of the prelimbic cortex and a recording electrode also in layer II/III to generate input/output curves. All experiments were carried out under protocols approved by the Institutional Animal Care and Use Committee. Our results showed alterations in performance on a working memory task and evidence of shifts in excitatory/inhibitory balance in the vHPC-mPFC circuit one week following TBI. Further research is needed to determine whether the observed changes in the mPFC could be an area of intervention for addressing long-term cognitive impairments after TBI.

64. ABSTRACT WITHDRAWN

65. Clot contraction drives structural redistribution of platelets, fibrin and red blood cells through energy minimization

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Blood clot contraction, volume shrinkage of the clot, plays a critical role in the restoration of flow past otherwise obstructive thrombi. Contraction is driven by platelet actomyosin forces and results in the compression of erythrocytes into a tessellated network in the core of the clot, leading to the terminology of polyhedrocytes. The aim of this work was to determine the mechanism driving the redistribution of the clot. Through the use of histology and scanning electron microscopy, we quantified the redistribution of platelets and fibrin to the exterior and erythrocytes into the core of the clot. Coupling experimental results with a poroelastic computational model of contracting clots allowed us to discover why this segregated distribution was more favorable than a homogenous distribution. As platelets begin to exert contractile forces and segregation results in the compression of erythrocytes into the core. As erythrocytes are compressed, they stiffen which effectively results in the platelets generating more contractile forces, furthering the compression of erythrocytes and redistribution of the clot components. The result is a lower energy for the overall system when compared to a homogenous clot distribution, revealing that energy minimization drives the structural redistribution of contracting clots. This has important clinical implications, since we have previously shown that patients with thrombotic conditions have impaired contraction compared to healthy subjects, pointing to a potential role of contraction in the obstructiveness and embologenicity of thrombi. Furthermore, this provides a potential basis for the decrease in efficacy of thrombolytic treatment with increasing time and has implications for the development of novel thrombolytics.

66. Structural mechanism for the mechanical rupture of fibrin blood clots

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Fibrin is a primary component of blood clots/thrombi and the major contributor to mechanical stability and integrity, including propensity for thrombotic embolization. We examined the mechanical failure of plasma clots with a linear crack (defect) at an edge with varying length, followed by strain-controlled stretching perpendicular to the crack. We quantified force-displacement and used scanning electron and confocal microscopy to assess changes in fibrin network structure around the defect. The mechanical response of the cracked fibrin gels could be separated into 1) non-linear regime (increasing slope);2) linear regime of elastic deformation (with constant slope); and 3) rupture regime (mechanical failure). Experiments were coupled with theoretical reconstruction of the force-displacement curves based on a mechanical model of the fibrin network. Length of the initial defect influenced the rupture process, where clots with a larger defect ruptured at up to 45% lower forces and 25% smaller displacements ($n=22$, $p<0.0001$). Rupture occurred as aligned fibrin fibers broke sequentially under the axial load, resulting in the remaining fibers being exposed to increasing loads and rupturing faster due to the positive cooperativity between fibers, meaning that as each fiber broke it was more likely the remaining fibers would break. Initial defect size was inversely related to the critical strain that influenced the rate of rupture, where smaller defects at higher strains propagated faster (increased cooperativity) (up to 10x). These results contribute to the understanding of how fibrin breakage underlies this embolization and the potential to influence the development of novel therapeutics targeting thrombotic embolization.

67. Investigating the lack of transferability of polygenic risk scores in cohorts with admixed ancestry

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Polygenic risk scores (PRS) summarize the results of genome-wide association studies (GWAS) into a disease risk. For some traits, PRS allows us to identify individuals with clinically actionable levels of risk. However, the majority of GWAS come from cohorts of European ancestry and predictive power is lower in non-European ancestry cohorts. Here we investigate the performance of PRS for height in admixed cohorts (African and European ancestry) to identify the causes for this pattern. We show that the predictive power of height PRS increases linearly with European ancestry (partial R2 ranges from 0.015-0.15 for 0-100% European ancestry). This effect persists with effect-sizes re-estimated using sibling pairs, ruling out residual population structure. The pattern also persists when PRS is computed using subsets of SNPs in regions of both high and low linkage disequilibrium (LD), indicating that LD differences are not the only cause. Frequency differences of associated variants between both ancestry backgrounds explain only up to 25% of the observed reduction in predictive power. Finally, we find no association between ancestry and phenotypic variance, indicating that there is no relationship between ancestry and genetic variance, and that the reduction in predictive power cannot be explained by causal specific to the African ancestry background. Thus, no single factor we investigated explains the difference in predictive power across ancestries, hinting that other factors, or a combination of multiple factors, are responsible for this pattern. This study highlights the need for more diversity in GWAS and a better understanding of the complexities of variant discovery/portability across cohorts and ancestries.

68. Epigenetic characterization of cardiac-specific endothelial cells

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Endothelial cells line all blood vessels in vertebrates, comprising the majority of non-blood cell types in the body. These cells contribute to whole-body nutrient distribution in a variety of ways, including regulation of local blood flow, regulation of trans-endothelial nutrient transport, and paracrine effects. Cardiac endothelial cells are the heart of this process – they are responsible for both supplying the myocardium and transporting freshly oxygenated blood to the rest of the body. In addition, cardiac endothelial cells share a developmental origin with their underlying parenchyma. They are derived from cardiac progenitor cells (CPCs), which can also give rise to cardiomyocytes and vascular smooth muscle cells. We characterize the accessibility landscape of cardiac endothelial cells at both steady state, and following exercise-induced hypertrophy and capillary expansion. Using the NuTRAP model to label nuclei of VeCadherin+ endothelial cells, we perform ATACSeq and RNASeq of flow cytometry-isolated endothelial nuclei. We show that cardiac endothelial cells retain epigenetic memory of their CPC origin, exhibiting open chromatin around binding sites for cardiac transcription factors, as well as in cardiomyocyte-specific genes. Finally, we show that this epigenetic memory is lost following culture, suggesting active maintenance of cardiomyocyte-specific open chromatin in endothelial cells.

69. A pooled in vivo CRISPRi screening for identifying functional partners of MYC in hepatocytes

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Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death and has limited treatment options. MYC is one of the most prominent oncogenes and is commonly active in HCC through amplification or overexpression. Genetic evidence shows that switching off MYC results in sustained tumor regression, indicating that targeting MYC with drugs could be a viable cancer therapy. Unfortunately, MYC is still “undruggable,” since it is an intracellular non-enzyme transcription factor. We hypothesize that MYC needs to cooperate with other factors to drive tumor growth, so targeting “druggable” functional partners would be an alternative way to inhibit MYC. Here we designed a first ever in vivo pooled CRISPRi screening for identifying MYC functional partner genes, which are required for MYC induced hyperproliferation and tumorigenesis in hepatocytes. Starting from 531 proteins identified to physically interact with MYC using proteomics, we constructed a Sleeping Beauty transposon system based library containing Fah, Myc CDS and sgRNAs against the 531 genes. We injected the library to Fah-/-;dCas9+ mice through hydrodynamic injection. After 4 weeks selection, the Fah+;Myc+ cells repopulated 90% of the liver. Through measuring the abundance of each gRNA by high-throughput sequencing, we identified several genes involved in spliceosome and cell division that are required for MYC driven hepatocytes proliferation. A lot of these genes showed activity in our screening, which is also reported by the literature. Thus, we established a reliable in vivo CRISPRi screening system and identified several potential targets for treating MYC driven HCC.

70. Modifying regulators of heterochromatin to improve reprogramming

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Liver transplantation is the only curative treatment for many liver diseases, including hepatocellular carcinoma, but transplantable liver tissue is limited. Hepatocytes, the primary liver cell type, derived through direct conversion of fibroblasts (hiHeps) present a promising approach for transplantation, but they are functionally incomplete. Heterochromatin restricts cell identity by preventing activation of alternative lineage genes by ectopic transcription factors, leading to incomplete activation of the target transcriptome. We determined the heterochromatin property of sonication resistance to be highly predictive of the genes which resist activation during reprogramming. We identified 172 proteins associated with sonication resistant H3K9me3 heterochromatin (srHC) by mass spectrometry. These srHC proteins were enriched for RNA binding domains and structurally disordered domains associated with phase separation. We hypothesize that depleting srHC proteins during reprogramming will render genes permissive for activation and enable expression of the target transcriptome. To identify srHC proteins impeding hiHep reprogramming, we conducted an siRNA screen of 103 srHC proteins during reprogramming and assessed the impact on expression by RNA-seq of hepatic genes in srHC domains and not activated in hiHeps. This revealed srHC proteins as key repressors of srHC genes. Depletion of many of the srHC genes activated overlapping, but heterogenous groups of srHC genes; revealing a highly complex landscape of heterochromatin regulation. In particular, depletion of one protein enhanced activation of > 20% of srHC H3K9me3 marked hepatic genes. These transcriptional changes were accompanied by global and local effects on histone modifications.

71. Quantifying genetic and phenotypic variability associated with protein features

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Characterizing the impact of genetic variants is not only important for defining the etiology of complex traits and diseases, but identifying new drug targets as well. Due to their clear potential for impacting a phenotype, coding regions are often targeted first when searching for disease causing variants or potential pharmacogenetic interactions. More specifically, genetic variants impacting functional and structural domains (i.e. active sites, DNA-binding domains, and amino acid modification sites) have known connections to explaining the variance among traits and diseases. 99.9% of the 20,396 human-reviewed proteins entered into UniProtKB/Swiss-prot database have at least one structural or functional domain and recently the positions of these domains have been mapped to the genome. We hypothesize, that there may be different amounts of selection within these regions and utilizing protein domain information will improve our ability to understand the relationship between genetic variation and phenotypes. We will use BioBin, a program for rare variant binning, to count low-frequency and rare variants in disordered regions using 1000 genomes data. By comparing the ratio of non-synonymous to synonymous SNVs at a global level in these regions we can capture the substitution rate that occurs across the genome. To identify diseases that are associated with each type of protein region, a phenome-wide association will be performed using whole-exome sequencing data from UK BioBank. This study presents a new strategy for studying the genetic etiology of disease by investigating the utility of protein domain information. In the future, we will map low-complexity regions of proteins to their genomic coordinates in order to have a more complete picture connecting genetics, protein structure, and phenotypic variability.

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