Quantifying effects of Alzheimer's disease on the human hippocampus using an ex vivo atlas combining MRI and histology

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There has been increasing interest in detailed hippocampal subfield morphometry in cognition, aging and disease research using in vivo MRI. However, research on in vivo morphometry is hampered by lack of a definitive reference model describing regional effects of aging and disease pathology on the hippocampus. Histological studies provide limited reference information due to their 2D nature and use of a measure like cell count rather than volume or thickness. We therefore built a 3D probabilistic atlas of the hippocampus combining post-mortem MRI with histology to serve as a reference for in vivo morphometry and to investigate hippocampal anatomy in Alzheimer's disease (AD). 0.2x0.2x0.2 mm^3 9.4T MR images from 31 ex vivo hippocampal specimens (13 normal controls (NC), 18 cases with a clinical diagnosis of dementia; of which 9 AD; mean age at death: 75 years) and histological sections at 0.2 mm intervals from 9 of the 31 specimens were combined into a probabilistic atlas of the hippocampus. All subfields were significantly smaller in AD compared to NC after age correction, with the largest decrease of ~40% in CA1 and stratum radiatum lacunosum moleculare (SRLM). T-statistic maps reveal that SRLM is affected throughout its length but show localized effects in the grey matter in middle/posterior regions. This probabilistic post-mortem atlas of the hippocampus allowed us for the first time to investigate AD-related effects on hippocampal subfield morphometry in 3D. These results support the hypothesis of differential involvement of hippocampal subfields in AD, providing further impetus for studying hippocampal subfields in relation to aging, disease and cognition during life.

Target Wnt/β-catenin to de-transform tumor vasculature and block glioma progression

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Aberrant vascularization is a hallmark of cancer progression and treatment resistance. Newly formed tumor blood vessels deliver oxygen and nutrients and produce paracrine factors to tumor microenvironment, fueling tumor growth, progression and metastasis. Targeting endothelial cells (ECs) has emerged as a fundamental strategy for cancer treatment. However, inefficient eradication of tumor-associated ECs remains a major barrier for current anti-vascular therapy. Here, we show that ECs transform to mesenchymal stem cell (MSC)-like cells, leading to EC chemoresistance in glioblastoma multiforme (GBM). Our analysis with human patient-derived ECs shows that GBM-associated ECs are resistant to treatments with chemodrugs including temozolomide (TMZ), etoposide and doxorubicin. Likewise, utilizing EC lineage tracing in a genetic murine GBM model, we show that EC-originated cells exhibit robust resistance to TMZ chemotherapy *in vivo*. Transcriptome analysis by deep sequencing reveals that ECs undergo mesenchymal transformation and stemness-like activation in GBM microenvironment. Furthermore, we identify an HGF/c-Met-mediated axis that induces β-catenin phosphorylation at Ser675 and Wnt signaling activation, inducing multidrug resistance-associated protein (MRP)-1 expression and leading to EC transformation, stemness-like activation, and chemoresistance. Finally, genetic ablation of β-catenin in ECs overcomes GBM tumor resistance to TMZ chemotherapy. Combination of Wnt inhibition and TMZ chemotherapy eliminates tumor-associated ECs, inhibits GBM growth, and extends mouse survival.

These findings illustrate a novel mechanism controlling therapy resistance in tumor-associated ECs, and suggest that de-transformation of ECs may provide an efficient strategy for anti-vascular and vessel normalization therapies in GBM, and possibly other malignant tumors.

Machine learning reveals genetic determinants of platelet traits

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Platelets play critical roles in hemostasis, inflammation, and wound healing. Better understanding the formation and function of platelets and their precursor megakaryocytes (MKs) may yield novel therapies for thrombocytopenia and ameliorate bleeding risk. Genome wide association studies (GWAS) previously linked single nucleotide polymorphisms (SNPs) with altered platelet size and/or count, but causal SNPs at most of these loci remain unknown.

We used machine learning to investigate biological mechanisms underlying platelet trait variation. Using the least absolute shrinkage and selection operator (LASSO), we created a quantified statistical model incorporating 9 chromatin features, including key MK transcription factors GATA1 and FLI1, to quantitatively estimate the probability a given SNP functionally impacts platelet trait variation. High-scoring SNPs associated with MK-related genes and molecular pathways, including variants known to impact MK development and/or platelet traits. Further, we identified several novel genomic loci with plausible biochemical mechanisms of action, including SNPs at the *TPM1* gene locus. Genetic *TPM1* ablation in human pluripotent stem cells altered MK development, increasing MK yield from progenitors.

In summary, we successfully applied machine learning to large-scale genetic data and identified important chromatin features for megakaryopoiesis. In addition to several known expression quantitative trait loci (eQTL) for platelet genes and/or traits, our model suggests several interesting loci as potential targets for further study. Cell-based investigation validated *TPM1* as a genetic regulator of megakaryopoiesis, and suggested that *TPM1* manipulation holds therapeutic utility for *in vitro* MK and platelet generation.

Tissue engineered nigrostriatal pathway as an anatomically-inspired test-bed for evaluating axonal pathophysiology

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Selective loss of long-projecting neural circuitry is a common feature of neurodegenerative diseases, such as the vulnerable nigrostriatal pathway in Parkinson's disease (PD). Current *in vitro* approaches for studying disease development generally do not mimic complex anatomical features of the afflicted substrates. Therefore, we apply this system as a biofidelic test-bed for evaluating axonal pathway development, maturation, and pathophysiology. Tissue engineered nigrostriatal pathways were formed by seeding dopaminergic neuron aggregates from the ventral mesencephalon of rat embryos at one end of hollow hydrogel micro-columns with a central extracellular matrix. Several days later, medium spiny neuron aggregates from the striatum were seeded on the other end for integration. Immunocytochemistry (ICC) and confocal microscopy were used to assess cytoarchitecture, synaptic integration, and mitochondrial dynamics. Dopaminergic micro-columns resulted in axonal extension >5mm by 14 days *in vitro*. ICC confirmed the appropriate neuronal sub-types in the two aggregate populations, collectively mimicking the general cytoarchitecture of the *in vivo* nigrostriatal pathway. Mitochondria along axonal tracts revealed dynamic intra-axonal mitochondrial motility in this system. Ongoing studies are evaluating real-time mitochondrial dynamics and axonal physiology under both baseline conditions as well as following the addition of exogenous α-Syn fibrils to model synucleinopathy in PD. This micro-tissue engineered nigrostriatal pathway recapitulates long-projecting axonal tracts with three-dimensional structure and multicellular composition, thus providing an anatomically-inspired platform that structurally and functionally emulates the nigrostriatal pathway *in vivo.*

A novel biomarker panel detects pancreatic ductal adenocarcinoma circulating tumor cells

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Pancreatic ductal adenocarcinoma (PDA) has a dismal 5-year survival rate of 7.7%. Lack of effective screening tools results in the majority of patients presenting with advanced disease. Circulating tumor cells (CTCs) are rare cells shed into the blood from solid tumors. Interrogation of CTCs from patient blood allows for a non-invasive approach of diagnosis and disease monitoring. Current PDA CTCs detection techniques are limited by poor sensitivity. In this study, CTCs from the genetically engineered autochthonous KPCY (*Kras;p53;Cre;YFP* alleles) mouse model of PDA were isolated and characterized using a novel flow cytometric approach that integrates CTC isolation with whole transcriptome analysis (WTA). Genes that were highly differentially expressed between YFP+ CTCs and CD45+ WBCs were combined in a multi-color flow panel to detect CTCs. Using this panel, we were able to detect 75-100% of CTCs from KPCY mice with pancreatic tumors. Index-sorting allowed us to correlate high content cytometric phenotype of each sorted cell with its molecular profile. Unsupervised hierarchical clustering revealed distinct transcriptional profiles in CTCs, including an 'epithelial' subtype (E) composed of cells expressing high levels of *Epcam, Cdh1, Muc1*, and cytokeratins, as well as EpCAM protein, a 'mesenchymal' subtype (M) in which cells have high expression of mesenchymal transcripts such as *Vim* and *Fn1* and a hybrid subtype with coexpression of mesenchymal and epithelial transcripts. This study will thus generate a novel PDA specific biomarker panel that can be used to detect and monitor disease progression of pancreatic cancer. In addition, whole transcriptome analysis of matched CTC and PDA tumor will provide unprecedented insight into CTC biology.

Influence of the gut microbiota on histone acetylation through butyrate oxidation

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The gut microbiota is a diverse microbial community that inhabits the human colon and accomplishes functions related to host defense, digestion, and immunoregulation. Given that numerous conditions, such as inflammatory bowel disease (IBD), are associated with a disrupted microbiota, investigating the interactions that occur between the microbiota and host is important for understanding disease pathogenesis and devising effective therapeutics. One mode of interaction involves small molecule metabolites, such as butyrate. Butyrate is known to inhibit histone deacetylases, which represent one of the many chromatin-modifying enzymes that regulate gene expression by controlling histone modifications. Using quantitative mass spectrometry, we have observed that mice lacking a microbiota have reduced levels of histone acetylation in the colon, possibly because of unopposed HDAC activity in the absence of butyrate. However, the loss of acetylation may also stem from less metabolism of butyrate to acetyl-CoA, the necessary cofactor for acetylation, in colonocytes. Through isotope tracing analyses performed in cell culture and mice, we have demonstrated that butyrate and inulin, a fermentable plant polysaccharide, provide carbon for histone acetylation reactions. Ongoing work is focused on mapping the genomic locations of reduced acetylation in germ-free mice and the consequent effect on gene expression, determining the relative contribution of butyrate to histone acetylation as an HDAC inhibitor versus a source of acetyl-CoA, and analyzing acetylation levels in IBD. Overall, our findings will advance insight into how the microbiota influences host cell epigenetics and gene expression programs, which may be relevant to the pathogenesis of inflammatory disorders like IBD.

4D-DIRECT PET reconstruction of parametric images using the Patlak Graphical Method

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Dynamic (4D) positron emission tomography (PET) is a nuclear medicine imaging technique applied in fields such as oncology, cardiology, neurology, and psychiatry. Typically, a set of 3D images are reconstructed as a function of time, to obtain the spatiotemporal distribution of a radiotracer in specific regions of interest in the human body. From this distribution, tracer kinetic models can be applied in order to convert the time-course of the radiotracer to images of biological interest (e.g. glucose metabolic rate related to constant K_i) which find applications in areas such as oncology or neuropsychiatry. Conventionally, each 3D image is reconstructed separately without taking into account the temporal relationship between each of them, and then tracer kinetic modeling is applied. However, techniques which include kinetic models within the 4D image reconstruction framework have been shown to directly deliver images of biological interest (i.e. without the need for post reconstruction kinetic modeling) with better signal-to-noise ratio due to more accurate modeling of the noise in the raw PET data. In this work, we implemented such a technique using the Patlak Graphical method [1] as tracer kinetic model (where K_i is the parameter of interest) within the DIRECT PET image reconstruction framework [2]. This technique was applied on a set of 2D+time simulations of a brain phantom. The results obtained not only show improvement in image quality, but deliver considerably lower residual sum of squares in K_i estimates when including the Patlak fit within the reconstruction algorithm compared to the conventional post reconstruction method. This preliminary work paves the way for the full 4D implementation and evaluation of dynamic approaches within the DIRECT framework.

Pathway level codon bias among synonymous rare variants is associated with Alzheimer's disease imaging biomarker

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Neuroimaging data can be utilized along with genetic information to improve our ability to detect Alzheimer's disease (AD) and characterize its pathogenesis. Though synonymous codons synthesize the same amino acid sequence, synonymous variants have been implicated in a number of diseases including neurological, cancer, heart, and more specifically AD. A growing body of evidence suggests variation among synonymous codons leading to codon bias can impact protein abundance, conformation, and function. One form of codon bias can be due to synonymous codon frequency, while making more or less optimal contacts with tRNAs can be another form. Furthermore, the 5' and 3' ends of genes can be biased with different synonymous codons. Although rare variants within specific pathways have been shown to be associated with AD, it remains unclear how pathway specific rare variants that affect codon bias are implicated in the disease. An association test between rare variants in pathways from whole-genome sequencing data and an AD-related neuroimaging phenotype (e.g. entorhinal cortex thickness) was performed using SKAT-O. Rare variants that affect codon optimality towards the 3' ends of genes in the p53 signaling pathway were associated with the imaging phenotype (FDR < 0.05). Backward elimination was used to identify the genes that have the strongest influence on the association for each pathway. While previous work has pointed to a connection between p53 and AD, this study is the first to suggest they are linked via rare variants in synonymous codons. Moreover, codon bias that affects certain regions of genes may play a role in the pathogenesis of AD and can be used to improve statistical power when performing pathway-based association tests.

Single cell resolution reveals cellular diversity and major heterotypic interactions in pulmonary vasculature

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Normal functioning of pulmonary vasculature is indispensable for maintaining physiological gas exchange and support of life. Furthermore, an increasing number of studies is linking structural and functional abnormalities in pulmonary vasculature with initiation, progression or severity of major lung diseases. However, a comprehensive mapping of various cell types in this lung niche is currently lacking.
Known and novel cell subpopulations were identified in dissected human or mouse pulmonary vasculature using single-cell RNA sequencing (scRNA-Seq) and unbiased clustering and dimension reduction analysis. Transcriptome profiles characteristic for each cell cluster were further analyzed for the expression of soluble factors and membrane receptors or cell surface markers. Initial analysis of clusters' signalome/receptome implied a major role for PDGFR, BMP and NOTCH signaling in vascular homeostasis. Additional crosstalk between clusters was observed in the case of expression of inflammatory mediators in resident cells and vascular function-modulating factors in inflammatory cell clusters. Analysis of gene products localized to cell membrane was used to establish cluster-specific panels of cell surface markers. An optimized antibody-staining panel and sorting of specific subpopulations was applied to gain a deeper sequencing and clearer differentiation in transcriptomes between and within selected clusters. The current study provides the first systematic insight into cellular composition and major functional pathways in adult pulmonary vascular compartment.