

ANTICIPATED RESULTS: Progenitor markers are related to extracellular matrix, eg DCN (logFC progenitors vs other cells types=3.17, expressed in 99.7% (pct.1) of progenitors, 26.1% (pct.2) of others). Endothelial and pericytes shared markers like RBP7 (logFC=1.67, pct.1 0.88, pct.2 0.39); pericytes also showed unique markers, eg RGS5 (logFC=2.29, pct.1 0.89, pct.2 0.17). Progenitors are further divided into 11 sub-clusters, one of which showed enrichment of CD36 (high proliferation potential), FABP4 (differentiation), and of the novel marker PALMD (logFC=7.13, pct.1 0.94, pct.2 0.48). All $p < 10E-5$. GSEA analysis suggests that inflammatory pathways are downregulated in both adipose progenitors and endothelial/pericyte cells in the femoral compared to the abdominal depot. DISCUSSION/SIGNIFICANCE OF FINDINGS: Single cell RNA sequencing provides unique insights into the molecular profile of cell types and the identification of novel subsets of cells within the human adipose tissue. Such cellular heterogeneity may explain differences in adipose function between individuals and eventually in the risk of obesity-associated metabolic diseases.

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Differential chromatin accessibility at dorsal root ganglia enhancers is associated with nerve injury

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ABSTRACT IMPACT: Our improved understanding of the changes in chromatin accessibility that occur in persistent pain states may identify regulatory genomic elements that play essential roles in modulating gene expression in the DRG. OBJECTIVES/GOALS: Efforts to understand genetic variability involved in an individual's susceptibility to persistent pain support a role for upstream regulation by epigenetic mechanisms. Our objective was to examine the transcriptomic and epigenetic basis of persistent pain following nerve injury. METHODS/STUDY POPULATION: We used a multiomic approach to identify novel molecular pathways associated with nerve injury-induced pain hypersensitivity. Adult Sprague Dawley rats were randomized to Chronic Constriction Injury (CCI) to the sciatic nerve or no treatment (naive). The ipsilateral L4-L6 dorsal root ganglia (DRG)s were removed on Day 14 and used for ChIP-seq for H3K4me1, ATAC-seq, and RNA-seq. We assessed for differential chromatin accessibility, transcription factor motifs, and enrichment for biological processes in chromatin accessible regions associated with cis-regulatory regions identified by ATAC-seq and H3K4me1 enrichment. Luciferase assays determined the functional significance of these sequences. RESULTS/ANTICIPATED RESULTS: We identified 58,446 genomic regions where H3K4me1 enrichment overlapped with chromatin accessibility. Differential analysis identified 2145 of these 58,446 regions that had changes in accessibility after CCI. The majority of these regions were located in introns or intergenic regions. Functional annotation of the differentially accessible regions identified disparate molecular functions enriched following nerve injury which suggests that altered

chromatin structure plays a role in the development of mechanical hypersensitivity. Motif analysis identified specific transcription factor families whose binding sequences were enriched in regions of increased or decreased accessibility. Luciferase assays showed significant enhancement or repression of gene transcription. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our data provides a comprehensive map of chromatin accessibility changes in the DRG after CCI and emphasizes the importance of chromatin structure in the development and maintenance of chronic pain.

77232

The role of the bromodomain and extra-terminal motif (BET) family of proteins in head and neck cancer tumorigenic phenotype

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ABSTRACT IMPACT: Understanding the biology underpinning head and neck cancer invasion and metastasis could lead to novel targeted therapies that are both effective and tolerable for patients with this debilitating disease. OBJECTIVES/GOALS: The bromodomain and extra-terminal (BET) family of epigenetic regulators has been implicated in the tumorigenesis of various cancers. In head and neck squamous cell carcinoma (HNSCC), the majority of morbidity is due to invasion and metastasis, so there is special interest in understanding the development of these phenotypes in HNSCC cells. METHODS/STUDY POPULATION: Stable SCC9 knockdown cell lines were generated by infecting cells with a lentivirus encoding Cas9-KRAB and a lentivirus encoding gRNA targeting each of the candidate BET proteins: BRD2, BRD3, BRD4, and BRDT. Knockdown was confirmed by qRT-PCR. Next, standard assays for proliferation (CellTiter-glo), invasion (Matrigel), and migration (scratch-wound healing assay) were performed for all candidate knockdowns and compared to a non-target control. RESULTS/ANTICIPATED RESULTS: Proliferation assay results revealed that BRD4 knockdown had a significant negative effect on the proliferative capacity of SCC9 cells in vitro. Similarly, BRD4 knockdown SCC9 cell lines were less invasive and less migratory. Interestingly, knockdown of BRD2, BRD3, and BRDT had no effect on proliferation, invasiveness, or migration. DISCUSSION/SIGNIFICANCE OF FINDINGS: We have identified BRD4 as a key driver of the HNSCC tumorigenic phenotype. In the future, we plan to investigate the role of JQ1, a pan-BET inhibitor, on HNSCC cell phenotypes. Additionally, we will identify the downstream targets of BRD4, which may serve as potential therapeutic targets for both HNSCC as well as other cancers more broadly.

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Multiple epidemics of multidrug-resistant tuberculosis revealed by spatial disease mapping and whole-genome sequencing analysis in urban China

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ABSTRACT IMPACT: Our study will integrate state-of-the-art methods in pathogen genomics, epidemiology, and geospatial analysis to identify both host- and pathogen-factors driving the MDR-TB transmission and the study outcome can inform the design of

targeted interventions **OBJECTIVES/GOALS:** The emergence of multidrug-resistant tuberculosis (MDR-TB) poses serious challenges for the global eradication of tuberculosis. Recent research has shown that transmission is now the dominant driver of MDR-TB. However, our limited understanding of where and among whom MDR-TB is transmitted hampers efforts to control person-to-person spread. **METHODS/STUDY POPULATION:** We used several analytic approaches to characterize the dynamics of MDR-TB transmission in Shanghai, China. We identified all culture-confirmed MDR cases between 2009-2016 in the city and 1) estimated individual-level risk factors for MDR disease; 2) mapped the TB cases by their home addresses and used a Bayesian spatial disease mapping method to identify regions with an elevated risk of MDR-TB; and 3) we sequenced all MDR isolates to understand whether transmission explained variance in risk that was not attributable to the distribution of individual or location-specific risk variates. **RESULTS/ANTICIPATED RESULTS:** There were 1034 MDR-TB cases among 16,315 culture-confirmed TB cases during the study period. Bayesian disease mapping identified spatial heterogeneity of MDR-TB and determined four hotspots with an elevated risk of MDR-TB, none of which were fully explained by individual or regional-covariates (Figure 1). Sequencing revealed that more than 40% of the MDR-TB strains were in genomic clusters, indicating recent MDR-TB transmission. Most importantly, MDR-TB cases in three of the four large clades (>8 isolates) were spatially concentrated in three strain-specific hotspots (Figure 2). **DISCUSSION/SIGNIFICANCE OF FINDINGS:** With the combination of traditional epidemiological tools, geographical, and genomic methods, this study revealed multiple loci of transmission of specific MDR-TB clades within a single city. Identification of where and among whom MDR-TB is transmitted can inform the design of targeted interventions.

Evaluation

27042

Pressure-pain thresholds at baseline and in response to isometric exercise in Achilles tendinopathy

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ABSTRACT IMPACT: Baseline presentation in AT (higher upper trapezius PPT with no difference at calf or tendon) may suggest a mechanism for persistent symptoms: with more advantageous central pain processing and no tradeoff peripherally, they may choose to continue their usual activities without regard for further damage to the affected tendon. **OBJECTIVES/GOALS:** Exercise-induced hypoalgesia, a reduction in pain with exercise, is often observed in healthy populations but is not well established in Achilles tendinopathy (AT). The aim was to compare pressure-pain threshold (PPT) at baseline and after fatiguing isometric exercise in AT and healthy controls. **METHODS/STUDY POPULATION:** 21 participants were recruited for the study: 7 AT (26.5 ± 8.8 yrs), 14 control (22.1 ± 3.2 yrs). After a familiarization session, participants completed an experimental session that involved performance of intermittent maximal voluntary isometric contractions (MVICs) (2s:2s duty cycle) in a Biodex3 dynamometer (Biodex Medical, Shirley, NY) for 4 minutes. PPT was measured at the medial gastrocnemius (calf), Achilles tendon, and upper trapezius at baseline

and immediately following the fatiguing isometric task using a Somedic Algometer (Somedic AB, Sweden). Data are expressed as Mean(SD). Change in PPT is expressed as a percentage of baseline PPT. Units for PPT are kPa. A priori alpha was set to 0.05. **RESULTS/ANTICIPATED RESULTS:** There was no change in tendon or calf PPT following isometric exercise in AT (tendon: p=0.78; calf: p=0.76), while both increased (i.e., exercise-induced hypoalgesia) in controls (tendon: 9.5(17.8), p=0.03; calf: 21.3(22.7), p<0.01). Neither group experienced a post-exercise change in upper trapezius PPT (AT: p=0.35; control: p=0.37). There was no between-group difference in baseline calf (p=0.14) or tendon (p=0.19) PPT. However, baseline and post-exercise upper trapezius PPT were significantly higher in AT (baseline: 335.6(194.8); post-exercise: 321.2(170.1)) than in controls (baseline: 193.7(75.1), p<0.01; post-exercise: 198.1(79.1), p<0.01). **DISCUSSION/SIGNIFICANCE OF FINDINGS:** These findings suggest: (1) in persons with AT, central pain processing is altered at baseline, but unaffected in response to isometric fatiguing exercise; and (2) in persons with AT, peripheral pain processing is unaffected at baseline, but is altered in response to this mode and dosage of fatiguing isometric exercise.

90449

Can Ultrasound detect changes to spinal cord blood flow before and after injury?

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ABSTRACT IMPACT: To track recovery and mitigate additional spinal cord injury (avoiding further paralysis), we are assessing the applicability of an implantable ultrasound device that can monitor the tissue health postoperatively. **OBJECTIVES/GOALS:** To date, no method has been developed that monitors spinal cord perfusion rate (mL/min/g) or pressure (mmHg) successfully after the surgery. Our goal is to design, construct, and validate (in animal models) a novel sensor that quantifies postoperative tissue perfusion in patients with SCI at the site of and downstream from the injury. **METHODS/STUDY POPULATION:** A sample size of 10 animals will allow us to test our hypothesis to track tissue perfusion before and after the SCI using ultrasound. After prepping and scrubbing the animal, the skin will be incised with a blade, bony structures will be removed and the spinal cord will be revealed. A 25-g weight will then be dropped from a height of 15 cm, and the animal will be observed for contraction of the lower extremities, a sign that the cord was damaged. Using Doppler ultrasound settings available on commercial transducers, we will investigate the acceptable frequency, as well as proper Doppler mode with and without contrast agents, and with and without elastography (stiffness mapping of the tissue). A range of frequencies will be tested (5 -25 MHz). **RESULTS/ANTICIPATED RESULTS:** It is expected that at frequencies 12 MHz and above, our radiologist collaborators would be able to easily detect the blood flow. It is also expected that the injury will have a noticeable effect on the changes of this detected blood flow. We aim to present figures demonstrating ultrasound image qualities obtained at various frequencies. We expect three such figures: one for gray scale ultrasound imaging, one for color Doppler and finally, one for spectral Doppler, which is the one mostly used to quantify blood flow. **DISCUSSION/SIGNIFICANCE OF FINDINGS:** To monitor recovery and mitigate secondary injury in patients with traumatic SCI, there is a need to monitor tissue perfusion intra-operatively. To address this need, we will design, construct, and validate a novel sensor that will postoperatively quantify tissue perfusion for the SCI patients at the site of the injury.