Center for Musculoskeletal Research (CSR)

9th Annual Musculoskeletal Research Symposium
Award-Winning Abstracts

Presenter: Audrie L. Langlais

Institution: MaineHealth Institute for Research **Department:** Center for Molecular Medicine

Poster Number: 29

Title: Morphine Decreases Extracellular Vesicles and miRNA Expression: Implications for Opioid-Induced Bone Loss

Co-Authors: Claire Morrow, Peter Caradonna, Kathleen Becker, Katherine J. Motyl

Abstract:
Opioids compromise bone health by reducing bone mineral density and increasing fracture risk. Previously, we identified morphine-induced bone loss in male, but not female, C57BL/6J mice, which was due to reduced bone formation. Bone loss was also associated with reduced circulating micro RNAs (miRNA) expression in serum and bone. However, the source of altered miRNA expression and the effects on bone formation have not been investigated. Based on opioids effects on the nervous system, and low opioid receptor expression in bone, we hypothesized that opioids alter the neural-skeletal axis to cause bone loss. To test this hypothesis, we treated 8-week-old C57BL/6J male mice with morphine (20 mg/kg, s.c., N = 5) or vehicle (0.9% saline, s.c., N = 5) for 1 hour and isolated serum extracellular vesicles that are released by cells to traffic cargo including miRNA/RNA. Within serum, the concentration of extracellular vesicles tended to be reduced by morphine ($p = 0.08$), suggesting decreased vesicle secretion contributes to changes in miRNA expression. A second cohort of C57BL/6J mice received an intratibial injection of Fast Blue, a retrograde neuronal tracer, and one week post injection dorsal root ganglion (DRG) which house the cell bodies of sensory neurons were isolated. Using in situ hybridization (RNAScope) we detected co-staining of Fast Blue+ and m-opioid receptor+ (Oprm1) sensory neurons, suggesting opioids may impact sensory nerves directly innervating bone, in addition to systemic effects as seen in serum. Lastly, we have generated an exosome reporter mouse, CD3emGFPl/s/l x Baf53bCre/+, and confirmed expression of CD63emGFP+ vesicles within bone that overlap with the neuronal marker βIII-Tubulin, suggesting their release from nerve terminals to recipient cells. Future studies will be expanded in reporter mice to test how morphine impacts neural-exosomes and miRNA contents in bone during bone loss, and more specifically osteoblast mineralization. This work will critically expand our understanding of how opioids impair bone and may influence future clinical treatment and prevention strategies.

Presenter: Stephanie L. Tsai

Institution: Massachusetts General Hospital **Department:** Center for Regenerative Medicine

Poster Number: 36

Title: Elucidating injury-site specific regenerative programs to rebuild the tendon

Co-Authors: Marie Noedl, Mor Grinstein, and Jenna L. Galloway

Abstract:
Tendons are essential connective tissues that transmit forces from muscle to bone. Their unique highly ordered, matrix-rich architecture is critical for proper function. Tendon injuries are common and frequently occur within the tendon or at the tendon-bone attachment. Current treatments are limited, costly, and variable in efficacy, leaving patients with impacted quality of life and altogether contributing to a growing economic healthcare burden. To date, the development of new therapeutic strategies has been hindered by the lack of experimental tendon regenerative models which may be leveraged to elucidate cellular and molecular mechanisms required for proper regeneration. While adult mammalian tendons can heal, tendon cells, or tenocytes, fail to respond and disorganized scar tissue with impaired function forms instead. Using lineage tracing and multiphoton imaging, we demonstrate that unlike their mammalian counterparts, adult zebrafish tenocytes can proliferate, migrate, and regenerate the tendon and tendon-bone attachment following full tear injuries. To investigate the molecular basis for zebrafish tenocyte plasticity, we performed single cell transcriptomics during homeostasis and regeneration in both injury models. We identify and characterize shared early transient injury-responsive cell-states which diverge during later stages into site-specific regenerative programs seemingly functionally driven by TGF-beta signaling in the tendon and Wnt signaling in the tendon-bone attachment. Finally, we present a spatial zebrafish tendon cell atlas and elucidate evolutionarily conserved cell populations. Our work debuts the adult zebrafish tendon as an invaluable regenerative model and opens avenues to generate genetic tools for performing cross-species comparative studies to uncover mechanisms driving regeneration versus fibrosis.

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Presenter: C R Coveney

Institution: Harvard **Department:** HEB

Poster Number: 22

Title: Joint morphology, osteoarthritis, and synergistic activity of GDF5 regulatory regions

Co-Authors: D E Maridas, P Muthuirulan, Z Liu, T Kahan, B L Proffen, A Kiapour , V Rosen, T D Capellini

Abstract:
Single nucleotide polymorphisms (SNPs) spanning a 130 kb interval containing GDF5 are associated with up to 1.8-fold increases in knee osteoarthritis (OA) risk and 1.6-fold increases in developmental dysplasia of hip (DDH) risk, among other cartilage disease phenotypes. Previous research on the cis-regulatory architecture of the GDF5 locus identified distinct GDF5 regulatory enhancers (R1–R5; GROW1) that control knee and hip cartilage gene expression, and harbor putative risk variants, including regulatory variants downstream of GDF5 (rs4911178 in GROW1; rs6060369 in R4). In vivo, each risk variant leads to morphological changes of the knee and hip joints, respectively, predisposing animals to cartilage degradation and spontaneous osteoarthritis development by one year of age (published). To explore the locus further, we examined other regulatory sequences for their effects on joint biology, including one termed R2 immediately upstream of the GDF5 5'UTR. We first found that deletion of R2 in vivo caused severe down-regulations of Gdf5 expression and significant phenotypic impacts, notably cartilage-driven morphological changes to the knee and hip by eight-weeks post-natal life. These anatomical domains partially overlap those effected by variants in R4 and GROW1. However, in contrast to R4 and GROW1 mutants, morphological changes as a result of the R2 deletion did not lead to increased disease risk (Fig 1a and 1b). Interestingly, in the 5'UTR of GDF5 there is also a commonly cited cis-regulatory variant, rs143384. To understand the impacts that that the "T" risk allele at this variant position has on expression and phenotype, we next engineered humanized single base-pair "T" replacement mice. Using allele-specific expression assays in mice we found that the risk "T" variant has minimal (i.e., statistically insignificant, p=0.12) effects on Gdf5 gene expression in each of the major forelimb and hind limb joints analyzed, yet results in only minor changes to joint morphology localized to the femoral plateau. This slight dysmorphology was not associated with any changes to cartilage thickness, or integrity (Fig 1c and 1d). In theory, this finding points to the importance of previously identified downstream regulatory variants in GROW1 and R4 as primarily causal for hip dysplasia and knee OA. However, as this 5'UTR variant resides next to R2 it could influence R2 activity in joints, and this is currently being assessed through phenotypic studies. Importantly, as the 5'UTR "T" allele at rs143384 resides on the same risk haplotype as R4 rs6060369 "T" and GROW1 rs4911178 "A" alleles it may also impact their activity through epistatic interactions. To assess this possibility, we finally tested in reporter assays in chondrocyte cells different combinations of risk and non-risk variants across the 5'UTR, R4, and GROW1 regulatory regions finding that synergistic interactions occur across the locus. These findings point towards complex genetic underpinnings of risk for different disease phenotypes at GDF5. Overall, our work provides an important developmental context to explain the mechanisms through which human GDF5 genetic variants can lead to joint disease.

Presenter: Shannon R. Emerzian

Institution: BIDMC **Department:** Orthopedic Surgery

Poster Number: 35

Title: Older Women with Longstanding Type 1 Diabetes Have Lower Femoral Strength and Region-Specific Deficits in Trabecular Bone Mineral Density of the Femoral Neck

Co-Authors: David C. Lee, Fjola Johannesdottir, I-Hsien Wu, John Gauthier, Surya Vishva Teja Jangolla, Marc Gregory Yu, Hetal S. Shah, George L. King, Tony M. Keaveny, Klaus Engelke, Elaine W. Yu, Mary L. Bouxsein

Abstract:
Type 1 diabetes (T1D) is associated with an increased risk of hip fracture, but the factors underlying skeletal fragility in older adults with T1D are not well understood. This study assessed regional differences in bone mineral density (BMD) and strength of cadaveric femora from postmenopausal women with longstanding T1D and non-diabetic controls.

Whole femora were acquired post-mortem from female Joslin Medalists with T1D ≥50yrs (n=11); age and sex-matched non-diabetic control femora were obtained from a tissue bank (n=10). Femora were scanned via axial computed tomography (CT, Siemens). CT scans were analyzed using Medical Image Analysis Framework (MIAF)-Femur, with cortical thickness (Ct.Th) and volumetric BMD (total=Tt; trabecular=Tb) assessed at the total hip (TH) and femoral neck (FN). FN volumes were further divided into quadrants: superior anterior (SA) and posterior (SP) as well as inferior anterior (IA) and posterior (IP). Femoral strength and DXA-equivalent TH and FN areal BMD (aBMD) and T-score were calculated using Biomechanical CT analysis (BCT, O.N. Diagnostics). Individuals were considered high fracture risk if they had either low aBMD (T-score≤-2.5) or fragile bone strength (≤3000N). The ratio of fall force to femoral strength was computed using a soft-tissue attenuated fall force and femoral strength from BCT. Wilcoxon rank sum tests assessed group differences; percent differences are between group medians.

The T1D group had an average (mean±SD) BMI=25.1±3.4kg/m2, HbA1c=8.3±0.9%, T1D duration=65±5yrs, age at onset=13±8yrs, and age at death=78±10yrs; age and BMI were not different between groups. 73% of T1D and 40% of controls were considered high risk (p=0.2). TH Tt.BMD and aBMD did not differ between groups. FN aBMD (p=0.07) and Tb.BMD (p=0.06) were lower in T1D (Table). Within the FN, women with T1D had Tb.BMD deficits that were largest in the SP (-35%, p=0.04) and SA (-35%, p=0.10) quadrants. Ct.Th did not differ between groups at any site. Women with T1D also had lower femoral strength (-26%, p=0.03), but similar fall force (p=0.25), resulting in a greater load-to-strength ratio (+28%, p=0.04).

These findings reveal deficits in femoral BMD and strength and greater load-to-strength ratio in older women with T1D, indicating increased susceptibility to hip fracture. As hip fractures may initiate in the superior FN, trabecular bone deficits in this region may contribute to the high risk of hip fracture in older adults with T1D.