Esophageal Tumor Cells
Ciclopirox Olamine Demonstrates Inhibitory Effects on Center
Dharmalingam Subramaniam, PhD2 and Scott Weir, PharmD, PhD2

METHODS: We analyzed the development of esophageal cell differentiation in colonoids in a co-culture system with proinflammatory macrophages. RESULTS/ANTICIPATED RESULTS: Our chronic inflammation models revealed an increase in proinflammatory macrophages present in the lamina propria and that these cells expressed significantly higher levels of notch ligand, Jagged1. Jagged 1 has been shown to enhance TH1 differentiation and T cells isolated from the mucosa of both chronic colitis models display strong TH1 skewing compared to controls. Chronic inflammation also contributes to intestinal barrier defects, enhanced permeability and bacterial translocation. We believe this enhanced intestinal permeability and subsequent bacterial translocation promote Jagged1 expression in intestinal macrophages. To support this concept, we show TLR stimulation induces the upregulation of Jagged1 in BMM. Additionally, the generation of BMM from our chronic DSS-induced colitis mice or age matched controls, revealed BMM derived from a host of chronic inflammation were skewed to a proinflammatory state prior to stimulation showing increased gene expression of several proinflammatory molecules including IL-1α, IL-1β, IL-12 and TNF-α. This would suggest monocytes migrating to the intestinal mucosa have more potential to become proinflammatory instead of traditional anti-inflammatory macrophages. Furthermore, proinflammatory notch ligand-positive macrophages co-cultured with colonoids, derived from unperturbed mice, significantly decreased the number of mucus producing goblet cells. In support of this observation, notch activation in intestinal stem cells promote absorptive (i.e. colonocytes) cell differentiation and prevents secretory cell (i.e. goblet cells) differentiation. DISCUSSION/SIGNIFICANCE OF IMPACT: Taken together, our results strongly suggest chronic inflammation modulates macrophage role in maintaining intestinal homeostasis through possible notch activation in both T cells and the intestinal epithelial barrier.

3508
Ciclopirox Olamine Demonstrates Inhibitory Effects on Esophageal Tumor Cells
Randi Ryan1, Shrikant Anant2, Prabhu Ramamoorthy, PhD2, Dharmalingam Subramaniam, PhD2 and Scott Weir, PharmD, PhD2
1University of Kansas Frontiers and 2University of Kansas Medical Center

OBJECTIVES/SPECIFIC AIMS: Drug repositioning has the potential to accelerate translation of novel cancer chemotherapeutics from bench to bedside. The goal of this study was to determine the effects of ciclopirox olamine (CPX) on esophageal tumor cells. METHODS/STUDY POPULATION: We tested the effect of CPX on four esophageal cancer cell lines, assessing cell proliferation and viability by MTT, alamarBlue, hexosaminidase and clonogenicity assay, respectively. We analyzed the effects of CPX on three-dimensional (3D) esophageal spheroids. We also analyzed effects on cell cycle by flow cytometry. For mechanism, we performed western blots for proteins involved in cell cycle regulation, apoptosis and the Wnt/β-catenin pathway. In vivo effects, we performed a murine xenograft model with intra-peritoneal administration of CPX (100 mg/Kg body weight daily). RESULTS/ANTICIPATED RESULTS: CPX inhibited growth of all cell lines in a time and concentration-dependent manner. CPX also inhibited growth of esophageal spheroids. Cell cycle analysis demonstrated G0/G1 arrest in cells treated with CPX. Western blot analyses demonstrated decreased expression of cyclinD1, CDK4, CDK6, and transcriptionally active β-catenin, supporting the role of CPX in cell cycle inhibition and decreased β-catenin activity. Finally, treatment of nude mice with CPX significantly decreased tumor xenograft volume. DISCUSSION/SIGNIFICANCE OF IMPACT: CPX demonstrates anti-tumor properties in esophageal cancer cell lines. The current results justify further research into the mechanism of this inhibition. Additionally, given its established safety in humans, CPX is a potential candidate for repositioning as an adjunct treatment for esophageal cancer.
and previous findings suggest a novel immune signaling mechanism by which a peptide fragment of high molecular-weight kininogen (HK-D5) acts as an accelerant of both innate and adaptive immune cell chemotaxis in multiple immune contexts. This has broad implications regarding a mechanism of immune-mediated inflammation in a variety of disease states, which might be amenable to the targeting this pathway for therapeutic intent.

**3013 Combined Annulus Fibrosus and Nucleus Pulposus Repair Prevents Degeneration in the Ovine Lumbar Spine**

Stephen Sloan1, Christoph Wipplinger1, Sirtac Kirnaz2, Rodrigo Navarro-Ramirez1, Antonella Schivinato1, Roger Härtl1 and Lawrence Bonassar1

1Cornell University and 2Fidia Farmaceutici

OBJECTIVES/SPECIFIC AIMS: The objective of this study is to assess the efficacy of combined AF and NP repairs to prevent degenerative changes and restore native disc morphology in an in vivo large animal model. We hypothesize that combined repairs will prevent disc degeneration following injury to a greater extent than the individual repairs after 6 weeks in vivo, as demonstrated through disc height measurements and disc morphology. METHODS/STUDY POPULATION: A total of 8 skeletally mature female Finn sheep were used in this study. Following a previously described method, IVDs from L1 to L6 of the lumbar spine were exposed using a lateral access, extraperitoneal approach. IVDs were randomized into 5 treatment groups: 1) intact discs, 2) discs injured via a 3 cm x 1 cm box annulotomy and partial nucleotomy, 3) injury followed by a high density collagen (HDC) AF patch, 4) injury followed by injection of a modified hyaluronic acid (HA) into the NP, and 5) injury followed by both the HDC AF patch and HA NP injection. The HDC treatment was 15 mg/mL type-I collagen mixed with 0.06mM riboflavin, injected at the defect site and crosslinked in situ with blue light. The NP injection was HA modified with C16 side chains to increase the viscosity of the hydrogel (HYADD 4®). At 6 weeks post-operatively, sheep were sacrificed and had 3T magnetic resonance images (MRI) taken of their lumbar spine. Disc height analysis and Pfirrmann grading were performed on each segment using MR images. Additionally, quantitative MRI analyses were performed using a custom MATLAB algorithm that segments NP from the surrounding tissue and directly measures the NP volume. ANOVA with Tukey’s HSD was used to determine statistical significance between groups for disc height and quantitative MRI analyses, and the Kruskal-Wallis test with Mann-Whitney tests was used to statistically analyze Pfirrmann Grades. All animal use followed approved IACUC protocol. RESULTS/ANTICIPATED RESULTS: As shown in axial MR images (Figure 1A), intact discs had hyperintense NP with a distinct border to the AF. The discs receiving injury with no treatment had hypointense NP with no distinct border between the AF and NP. Individual and combined treatment with the HA NP injection and HDC AF patch appeared to preserve the hyperintense NP signal and AF/NP border. Intact control discs were not degenerated and had an average Pfirrmann grade of 1 (Figure 1B), while injured, untreated discs had significant degeneration with an average Pfirrmann grade of 3. Discs receiving the HA NP injection and collagen AF patch individually showed fewer signs of degeneration than the injured alone, and the combined treatment resulted in the least amount of degeneration with Pfirrmann grades not significantly different than the intact controls. Disc height index confirmed the trends seen in the Pfirrmann grades (Figure 1C), where injured discs lost 20% of the intact disc height, the individual NP and AF repairs restored 5-10% of intact disc height, and the combined repairs preserved 90% of the intact disc height. The NP voxel count of all treatment groups were similar to the intact controls (Figure 1D). DISCUSSION/SIGNIFICANCE OF IMPACT: The objective of this study was to determine how combined AF and NP can prevent degenerative changes to the disc in a large animal in vivo model. Pfirrmann grading and disc height index results show that the greatest preservation of disc morphology was seen with combined AF and NP repairs, while the individual strategies prevented degenerative changes better than injury with no treatment. It appears the HA NP injection restores lost water content to the disc following injury, and the AF collagen patch plays a role in maintaining the NP repair within the disc. This is the first study to our knowledge to attempt combined AF and NP repairs in an in vivo large animal model. Combining NP and AF repairs leads to significantly improved outcomes following disc injury, which warrants the translation of combined repairs into the clinic to improve patient outcomes with degenerative disc disease involving NP and AF.

**3528 Common Mechanisms Underlying Epilepsy and Tauopathy**

Ryan Adam Cloyd1, Joe Abisambra2 and Bret Smith1

1University of Kentucky Center for Clinical and Translational Science and 2University of Florida

OBJECTIVES/SPECIFIC AIMS: Neurologic disorders are among the most significant health challenges facing society today. Although different neurologic disorders are often thought to be distinct from one another, evidence suggests similar processes may contribute to pathology in different diseases. Previous studies suggest that common disease mechanisms contribute to the development of epilepsy and tauopathy. The purpose of this study is to better characterize this relationship and explore potential therapeutic avenues to slow disease progress. METHODS/STUDY POPULATION: This study uses the pilocarpine-induced status epilepticus model of temporal lobe epilepsy to explore the effect of severe seizures on tau pathology. Brains were collected from mice at 6 or 24 hours after induced status epilepticus. Homogenates were analyzed via Western blot to look for changes in tau phosphorylation or activity of two major regulators of tau phosphorylation, GSK3β and PP2A. These data show that changes in tau phosphorylation dynamics occur at a much earlier time point after status epilepticus than has previously been described. RESULTS/ANTICIPATED RESULTS: GSK3β activity increased within 6 hours and remained elevated by 24 hours. PP2A activity initially decreased but returned to normal by 24 hours. These data show that changes in tau phosphorylation dynamics occur at a much earlier time point after status epilepticus than has previously been described. DISCUSSION/SIGNIFICANCE OF IMPACT: The current project supports previous observations that seizures promote tau phosphorylation in vivo, but suggests that changes begin much earlier than previously thought. Further work is needed to understand how post-seizure changes in tau phosphorylation develop over longer periods of time. Additionally, future work will characterize the effect of tauopathy on electrical activity in vivo and in vivo.