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The role of LSD1 in Chronic Kidney Disease
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Chronic kidney disease (CKD) affects more than 37 million Americans. One of the pathological features of CKD is fibrosis. Renal fibrosis is characterized by fibroblast activation, epithelial-mesenchymal transition (EMT), and excessive extracellular matrix (ECM) deposition. Renal fibrosis can lead to damage of the normal kidney structure and loss of function. The underlying mechanisms are not well understood, so I determined the possible effects and molecular mechanisms of LSD1 in renal fibrosis.

LSD1 inhibitor, GSK-LSD1, treatment in macrophages showed decreased LPS induced IKBα and p65 phosphorylation at the protein levels after 30 minutes. Additionally, GSK-LSD1 treatment ameliorated TGF- β induced EMT with increased E-cadherin and decreased SNAI1 in human tubular epithelial cells (HKC-8). Moreover, LSD1 also plays a role in fibroblast activation. In kidney fibroblast (49F) cells, TGF- β was used to induce fibrosis markers. The importance of LSD1 in the activation of fibroblasts was shown by decreased levels of FN, COL1, and α-SMA when 49F were treated with GSK-LSD1. Molecular mechanism analysis showed that p-Smad3 was decreased after GSK-LSD1 treatment, indicating that LSD1 induced fibrosis through the TGF-β/smad-3 signaling pathway. These results demonstrate that LSD1 plays a role in EMT and fibroblast activation.

Understanding the role of LSD1 in renal fibrosis is critical to dissecting the mechanisms of chronic kidney disease at the molecular level. Our data showed that LSD1 activation promotes the process of renal fibrosis and suggests that LSD1 can be a therapeutic target for chronic kidney disease.
MicroRNAs (miRNAs) are small non-coding RNAs that are key post-transcriptional regulators; and the activity of the Wnt-β-catenin pathway in bone can be suppressed by microRNAs such as miR-433-3p. The Wnt signaling pathway in bone involves the proliferation of β-catenin, which allows the TCF/LEF transcription factors to activate gene expression programs essential for osteoblast differentiation and function. We recently showed that miR-433-3p negatively regulates bone formation in vivo and canonical Wnt signaling in vitro. One recently identified miR-433-3p target is Rspo3, which promotes the activation of the Wnt signaling pathway. Using a candidate gene approach, we identified additional potential miR-433-3p targets in the Wnt pathway in the Lrp4 and Wnt16 3' UTRs. miR-433-3p complementary regions of each gene were amplified and cloned into the 3'UTR of the luciferase reporter plasmid pMiR-Report so their activity can be analyzed. C3H10T1/2 cells were transfected with each of the test genes as well as a miR-433-3p inhibitor or a non-targeting RNA inhibitor. An increase in luciferase reporter activity in cells transfected with a miR-433-3p inhibitor relative to non-targeting RNA inhibitor indicates that the region of interest is targeted by miR-433. Our data showed that Lrp4 and Wnt16 3' UTR are miR-433-3p targets, with Wnt16 3' and Lrp4 displaying a 2 and 1.5 fold respective increase in activity when miR-433-3p inhibitor is present. Observing which genes are targets for miR-433-3p and its effects on this pathway can increase the potential for micro-RNA inhibitor therapeutics as a possible strategy to manage bone loss disorders.
Angelman syndrome (AS) is a neurodevelopmental disease characterized by developmental delays, cognitive disabilities, seizures, speech impairments, and ataxia. It is caused by a mutation or deletion of the maternally inherited UBE3A allele (Hsiao). UBE3A is paternally imprinted through silencing with the UBE3A antisense (UBE3A-ATS) transcript, and studies show that reducing UBE3A-ATS activates UBE3A expression. One drug shown to reduce UBE3A-ATS is PHA533533, a kinase inhibitor with unknown targets. Before PHA533533 can be developed into a therapeutic approach, it is important to understand its mechanisms of action. Combining the knowledge that PHA533533 acts on kinases and that a cyclin-dependent kinase, CDK9, is responsible for phosphorylating RNA Polymerase II, it is hypothesized that PHA533533 inhibits CDK9 to reduce transcription of UBE3A-ATS, leading to increased UBE3A expression. To test this hypothesis, two CDK9 inhibitors, AZD4573 and BAY1251152, were used to treat neurons modeling AS at various concentrations. Their effect on UBE3A-ATS and UBE3A expression was compared to PHA533533. Both BAY1251152 and AZD4573 reduced the UBE3A-ATS and increased UBE3A expression in a dose-dependent manner, showing a similar trend to PHA533533. These results indicate that CDK9 inhibitors have similar effects to PHA533533, suggesting CDK9 inhibition is likely contributing to the activity of PHA533533. Although these CDK9 inhibitors show similar trends to PHA533533, the level of UBE3A expression is lower in magnitude compared to PHA533533. This suggests PHA533533 likely has other targets in addition to CDK9. Further research in understanding PHA533533’s targets may provide key information about the mechanisms responsible for transcribing UBE3A-ATS.

Angiogenesis is critical for the growth and metastasis of tumors and often forms abnormal, leaky vasculature that inhibit the permeability of chemotherapy drugs. The biomarker prostate-specific membrane antigen (PSMA) is a transmembrane protein present on prostate epithelial cells that increases expression during prostate cancer (PCa) progression and is also present on tumor vasculature. Previous studies show that PSMA positive PCa epithelial cells induce PSMA on the surface of PSMA-negative human umbilical vein endothelial cells (HUVECs). Therefore, in this study we explored how PSMA is upregulated on HUVECs. Exosomes are a type of extracellular vesicle that contain constituents (protein, DNA, and RNA) of the cells that secrete them. They are taken up by distant cells, where they can affect cell function and behavior, and are important in cancer progression. Here we show by immunofluorescence microscopy that exosomes isolated from PSMA-positive PCa cell media can induce PSMA expression on HUVECs. To confirm that exosomes were present in the cell media, exosomes were purified then analyzed by SEM. Presence of exosomes was confirmed by size and visible lipid bilayer. Additionally, CD63 and CD81 positive exosomes were shown by flow to be PSMA-positive and negative for Golgi contaminant GPR94 as shown by western. Finally, exosomes were labeled with celltracker-green and incubated with HUVECs overnight. Immunofluorescence microscopy confirmed the uptake of the stained exosomes and RT-qPCR revealed an increase in PSMA expression compared to the untreated. These findings suggest that PSMA positive PCa exosomes participate in the induction of PSMA on the surface of HUVECs.
Using CRISPRi to Study Persistence in Borrelia Burgdorferi and Leptospira interrogans.

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The spirochetes *Borrelia burgdorferi* (Bb) and *Leptospira interrogans* (Lin) are the causative agents of Lyme disease and Leptospirosis, respectively. Nearly a half million cases of Lyme disease are reported annually in the U.S. alone, and nearly 60,000 fatalities are reported from Leptospirosis annually worldwide. Although these diseases are widespread, the pathogenic mechanisms present in these bacteria are largely uninvestigated due to traditional methods for genetic manipulation being costly, inefficient, and time-consuming. Clustered regularly interspaced short palindromic repeats interference (CRISPRi) has been shown to be an efficient method for inducing gene silencing in order to study these pathogenic mechanisms. CRISPRi consists of a catalytically inactive Cas9 (dCas9) and short guide RNA (sgRNA), which contains a 20-bp complementary region that allows it to bind to and interfere with genetic elements. The goal of this project was to develop inducible CRISPRi constructs towards specific genes of known importance in Bb and Lin, like RpoS in Bb and PerRA/PerRB in Lin, such that the effects of silencing these genes on the persistence of the bacteria inside their host organisms can be studied effectively. To do this, base CRISPRi constructs were obtained from collaborating laboratories and adapted into plasmid vectors using PCR reactions to develop unique constructs for genes of interest. These plasmids were transformed into competent cells to be replicated and conjugated into Bb and Lin. From there, mice were inoculated and inhibition of the expression of key genes was induced in order to better understand the effects of these genes on persistence.
Invasion into the surrounding stroma is seen as a crucial step in endometrial cancer metastasis. It signifies an event where cancer cells have acquired the capacity to overcome an immense barrier before disseminating. In this study, we specifically explored the invasion of cancerous trophoblast cells into endometrial stromal fibroblasts. The interactions and cross talk between cancer and stromal cells highly suggest that fibroblasts become activated and adopt protumorigenic characteristics. To conduct this study, a set of genes were knocked out in stromal cells. A series of invasion assays were then performed using time lapse microscopy to monitor the cancer stromal interactions. Results were analyzed using cell profiling softwares, FIJI and Ilastik. FIJI was used to quantify the number of invasive forks, escaped trophoblast cells, and area of invasion; Ilastik was used to determine cell displacement, persistence, and velocity. The invasion assays showed how trophoblast cells responded to gene knockouts, in whether it increased or reduced the level of invasion when being compared to the controls. Image processing and data analysis can be applied to future assays to quantify different aspects of cancer stromal invasion.