Catalase comparable to the vitamin E treatment. DISCUSSION/SIGNIFICANCE OF FINDINGS: If addition of apelin causes an antioxidant response in all three cells types, this can build on evaluation of the APJ system as a therapeutic option for those with CKD and CRS4 to minimize both inflammatory and oxidative stress. With the data gathered here, we expect to recreate the results in a CKD rat model that highlights these same manifestations.

25380

Cholecystokinin-B Receptor Mediates Growth of Hepatocellular Carcinoma*

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ABSTRACT IMPACT: Cholecystokinin-B Receptor Mediates Growth of Hepatocellular Carcinoma with the use proglumide. Proglumide is a non-selective antagonistic drug therefore, strategies that block signaling at the CCK-BR may provide to be a novel therapeutic option for Hepatocellular Carcinoma treatment OBJECTIVES/GOALS: Cholecystokinin (CCK)and gastrin mediate the growth of Hepatocellular Carcinoma (HCC) through CCK-R and interruption of this signaling pathway could decrease HCC. CCK-Receptors are overexpressed in HCC and proliferation may be mediated through CCK-B. Blockade of the CCK-BR with proglumide decreased both growth in vitro and tumor growth in vivo. METHODS/STUDY POPULATION: RNA was extracted from murine Hepa1-6, RIL-175 and human HepG2 cells and was evaluated by qRT-PCR for expression of CCK-AR, CCK-BR and gastrin. CCK-R protein expression was analyzed by flow cytometry. HCC cells were treated in vitro with CCK peptide, the CCK-AR antagonist or the CCK-BR antagonist. Proliferation of selective CCK-R KO cells was compared to that of wild-type cells. To determine the effect of a CCK-R antagonist on tumor growth in vivo two cohorts of mice bearing subcutaneous Hepa1-6 or RIL-175 HCC tumors were treated with an oral bioavailable CCK-R antagonist proglumide or untreated water for 3-4 weeks. The mice bearing Hepa1-6 tumors were placed on a high-fat diet to raise blood CCK levels. Mice bearing RIL-175 tumors were fed standard chow to determine if proglumide could block autocrine growth by gastrin. RESULTS/ANTICIPATED RESULTS: The mRNA expression of CCK-AR, CCK-BR and gastrin were increased 80-90-fold in all HCC cell lines compared to that of normal liver. CCK-BRs were detected on >85% of the cells by flow cytometry. CCK peptide (1nM) stimulated HCC growth in vitro in both wild-type cells and in CCK-AR KO cells but not in CCK-BR KO cells. CCK-BR antagonist blocked CCK-stimulated growth in vitro but the CCK-AR antagonist did not, suggesting that the CCK-BR was responsible for mediating proliferation. In vivo tumor growth was significantly reduced with proglumide treatment by 70% ($p<0.05$) in Hepa1-6 and by 73% ($p<0.001$) in RIL-75 tumors, respectively. DISCUSSION/SIGNIFICANCE OF FINDINGS: CCK-Rs are overexpressed in HCC and proliferation appears to be mediated through the CCK-BR. Downregulation with CRISPR Cas9 or blockade of the CCK-BR with an antagonist decreases growth in vitro and proglumide therapy decreases tumor growth in vivo. Strategies that block signaling at the CCK-BR maybe a novel therapeutic option for HCC treatment.

27558

Obeticholic acid (OCALIVA ®) protects against 2,8-dihydroxyadenine nephropathy in mice

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ABSTRACT IMPACT: This work may lead to new treatments for crystalline nephropathies. OBJECTIVES/GOALS: This study investigated obeticholic acid (OCALIVA ®) as a potential treatment for 2,8-dihydroxyadenine (2,8-DHA) nephropathy using a mouse model. The treatment was investigated in both sexes at two timepoints. METHODS/STUDY POPULATION: Male and female C57BL/6J mice (12 weeks of age) were fed chow (Research Diets D19120401i) or chow admixed with adenine (0.2% w/w) ad lib for either 3.5 or 7 weeks. Mice were treated with either vehicle (corn oil) or obeticholic acid (10 mg/kg BW) by gavage 5 days per week. Each of the 16 combinations of sex/diet/timepoint/treatment groups had an $n = 6$ (96 mice in total). Food and body weights were measured twice per week, and 24-hour urines were collected prior to euthanasia. Serum and organs were collected and processed for biochemical and histopathological analyses. RESULTS/ANTICIPATED RESULTS: At both the 3.5-week and 7-week timepoints, dietary adenine robustly increased BUN and serum creatinine compared to control diet in vehicle-treated male and female mice ($P < .01$, all comparisons). At the 3.5-week timepoint, obeticholic acid reduced BUN in male ($P < .05$) but not female adenine mice. Obeticholic acid did not affect serum creatinine at this timepoint. At the 7-week timepoint, obeticholic acid reduced BUN in female ($P < .05$) but not male adenine mice. At the 7-week timepoint, obeticholic acid reduced serum creatinine in both male ($P < .05$) and female ($P < .01$) mice. Biochemical and histopathological analyses are ongoing, and we anticipate that the results will agree with the serum chemistries. DISCUSSION/SIGNIFICANCE OF FINDINGS: Obeticholic acid is FDA-approved for primary biliary cholangitis, and it is in clinical trials for several other hepatobiliary diseases. Although currently untested in humans, it is nephroprotective in many preclinical models of kidney disease. This study is the first to investigate obeticholic acid in a model of crystalline nephropathy.

32219

Differences in cell death in methionine versus cysteine depletion

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ABSTRACT IMPACT: Reducing methionine levels has repeatedly been shown to reduce cancer growth in vivo, while at the same time increasing lifespan in healthy animals. However, the mechanisms behind the beneficial effects of methionine restriction are currently unknown. OBJECTIVES/GOALS: We hypothesized that comparing the response of a cancer cell line to depletion of the amino acids methionine and cysteine would give us insight into the critical role of these two closely related amino acids in cancer, and help advance methionine restriction on the translational science spectrum. METHODS/STUDY POPULATION: We used the human

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melanoma cell line A101D to analyze the response to three conditions: methionine depletion, methionine replacement with homocysteine, and cysteine depletion in vitro. We measured proliferation/viability, gene expression patterns, and glutathione levels. We also assessed ferroptotic versus apoptotic cell death. We then used a normal human fibroblast cell line to compare responses. RESULTS/ANTICIPATED RESULTS: The transcription factors ATF4 and NRF2 were activated by all three tested conditions in melanoma cells. Glutathione levels were decreased by ~40% in cells grown without methionine, and by 95% in cells grown without cysteine. Lipid peroxidation was increased in cells grown without cysteine, but not in cells grown without methionine. Inhibiting ferroptotic cell death partially rescued proliferation in cysteine-depleted but not in methionine-depleted cells. Almost 70% of cells grown in methionine-depleted media stained positive for Annexin V, an indicator of apoptosis, compared to only 20% of cells grown in cysteine-depleted media. In normal cells, ferrostatin recued proliferation/viability to 86% of control levels in cysteine-depleted cells. Ferrostatin did not affect methionine-depleted normal cells. DISCUSSION/SIGNIFICANCE OF FINDINGS: These results indicate that methionine depletion leads to apoptosis, while cysteine depletion leads to ferroptosis. We found overlapping pathways activated by methionine and cysteine depletion at the gene expression levels, but divergences in cell death pathways ultimately activated.

32329

A novel mouse model of Ataxia Telangiectasia for testing small molecule readthrough compounds

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ABSTRACT IMPACT: Small molecule readthrough compounds are a promising therapeutic with the potential to overcome nonsense mutations thereby enabling the production of functional ATM protein in patients with Ataxia Telangiectasia OBJECTIVES/GOALS: To generate a novel mouse model of Ataxia-Telangiectasia for testing small molecule readthrough compounds that both expresses a clinically relevant nonsense mutation and recapitulates the major symptoms of the disease, including a progressive loss of motor coordination not previously observed in prior A-T animal models. METHODS/STUDY POPULATION: Using a double-hit strategy to increase genotoxic stress, we generated a novel A-T mouse model that expresses a clinically relevant (c.103C>T) mutation in the Atm gene and a knockout of the functionally related Aptx gene. We then characterized the mouse across multiple domains related to the various symptoms related to the disease. This includes examination of survivability, immunologic function, cancer prevalence, and motor behavior and its associated cerebellar dysfunction and atrophy. Lastly, we tested the ability of small molecule readthrough compounds to enable production of ATM from tissue explants extracted from these ATM deficient mice. RESULTS/ANTICIPATED RESULTS: The double mutant mice display reduced survivability compared to control mice (53% vs. 97%; $p<0.0001$), dying at a clinically relevant rate of about 30% from thymomas. At postnatal day 400 (P400), only AtmR35X/R35X; Aptx-/- mice, and none of the controls expressing at least one wildtype Atm or Aptx gene develop a motor behavioral deficits that are associate with reduced Purkinje neuron diameter (8.0 ±0.4 μm vs. 9.92 ±0.5; $p<0.01$) and density (4.3 ±0.2 vs. 6.0 ±0.3 per 100 μm; $p<0.05$) as well as cerebellar atrophy (cerebellum/forebrain area 0.26 ±0.01 vs. 0.31 ±0.01; $p<0.001$). ATM deficient mice also display disrupted thymocyte development and metabolic function. When exposed to small molecular readthrough compounds, greater than 50% of the ATM protein is restored. DISCUSSION/SIGNIFICANCE OF FINDINGS: We have created a novel, clinically relevant A-T mouse model that develops a severe ataxia associated with changes in cerebellar function and atrophy as well as demonstrate the potential of SMRT compounds as an A-T therapeutic.

33886

Deregulation of the LANCL2 Pathway in Alzheimer's Disease

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ABSTRACT IMPACT: My work focuses on understanding the role of an immunomodulatory pathway in Alzheimer's disease as a potential therapeutic target. OBJECTIVES/GOALS: My long-term goal is to develop effective drugs to halt the progression of Alzheimer's disease (AD). The overall objective of this translational work is to study the deregulation of the Lanthionine Synthetase C-like 2 (LANCL2) immunomodulatory pathway in AD. I hypothesize that stimulation of the LANCL2 pathway will inhibit progression of AD. METHODS/STUDY POPULATION: These studies use a transgenic rat model of AD, and a LANCL2-binding drug, BT-11, that crosses the BBB. Tg-AD Fisher 344 rats express human APP with the Swedish mutation and human presenilin-1 with the ? exon 9 mutation. Tg-AD rats develop age-dependent AD-like pathology. Rodent chow containing BT-11 (10mg/Kg bw) was administered orally from 5 months of age (pre-pathology) to 11 months of age. Eight groups of rats (8 rats/group) were included: WT and Tg-AD x 2 sexes x 2 conditions (BT-11 treated and untreated). Rats are analyzed for: (1) Spatial learning and memory with an active place avoidance test. (2) AD pathology including amyloid plaques and activated microglia, with hippocampal immunohistochemistry. (3) LANCL2-signaling and other AD relevant pathways, with hippocampaI western blot and RNAseq analyses. RESULTS/ANTICIPATED RESULTS: I predict that BT-11 will prevent and/or mitigate some aspects of AD pathology displayed by the AD transgenic rats (Tg-AD). Tg-AD rats treated with BT-11 should perform better in the spatial learning and memory tasks, than the untreated Tg-AD rats. I also expect that the immunohistochemical analysis will reveal reduced pathological hallmarks, including amyloid plaques and reactive microglia, in hippocampal tissue of BT-11 treated versus untreated Tg-AD rats. Through western blot and RNAseq analyses, I will establish how BT-11 treatment affects the LANCL2 signaling cascade as well as other AD relevant pathways, in treated and untreated rats from both sexes and both genotypes. DISCUSSION/SIGNIFICANCE OF FINDINGS: My proposed translational research will address the potential of targeting the LANCL2 pathway to improve AD treatment. Discovering the effects of stimulating the LANCL2 pathway on AD pathology is a novel approach in AD drug development. I expect that my studies with BT-11 will positively influence therapeutic outcomes of this devastating condition.

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