pathogenesis mechanisms necessary in diseases like myocarditis, without similar immunosuppressive risks as their pharmacologic counterparts. We obtained plasma from healthy men and women and isolated nanoparticles, which were analyzed for physiochemical markers of human EVs. After confirming presence of EVs, we injected these plasma-derived extracellular vesicles (PEV) into male BALB/c mice vs. PBS control intraperitoneally on days -1, 0, 1 of viral infection (day 0) in a highly translational, mouse model of myocarditis. Hearts were examined at day 10 at the peak of acute myocarditis, using standard histological and cell composition analyses. RESULTS/ANTICIPATED RESULTS: Mice treated with PEV had significantly less myocardial inflammation both histologically and by gene expression of immune markers in the heart. The immunoregulation by PEV treatment decreased many key components of innate immune response networks that are known to be upregulated during acute myocarditis: TLR4+ mast cells and macrophages and complement. These pathways drive the profibrotic gene and protein changes that lead to remodeling, fibrosis, and disease progression observed in patients. We anticipate that when we analyze later timepoints in this model (day 35 post infection) which are normally associated with development of chronic myocarditis, dilated cardiomyopathy, we will see reduction of this fibrosis and of damage-associated changes. DISCUSSION/SIGNIFICANCE: These data suggest that EVs from plasma may be a novel treatment for viral myocarditis, but translation of EVs is hindered by their heterogeneity. We demonstrate characterization both physiochemically and functionally in a well-defined model. Such practices are necessary as these contemporary products challenge current regulation standards.

426

Long non-coding RNA WNT5A-AS1 shows sex-dimorphic differences in survival for males with glioblastoma

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OBJECTIVES/GOALS: The goal of this study is to evaluate the role of WNT5A and WNT5a-AS1 in sex-differences of GBM progression. In our preliminary studies, we found that a long non-coding RNA WNT5A-AS1 is overexpressed in male GBM patients. We also found that WNT5A-AS1s expression shows a negative correlation with overall survival within male patients. METHODS/STUDY POPULATION: We will define the mechanism by which WNT5A-AS1 regulates WNT5a-mediated glioma stem cell (GSC) maintenance by assessing the effects of inhibiting WNT5A-AS1 expression on transcriptional activity and stemness in GSCs. We will determine if there are distinct Wnt-signaling patterns in male and female isogenic murine astrocytes by examining the expression of downstream proteins in the Wnt signaling pathway and how inhibition of WNT5A-AS1 alters this expression. We will then examine the impact of WNT5A-AS1 on temozolomide (TMZ) resistance in-vitro and in-vivo. We will assess the cell viability and survival of GBM PDX cells upon treatment with TMZ in vitro. Next, we will assess the capacity of knockdown of WNT5A-AS1 to increase sensitivity to TMZ-induced cell death and prolong survival in vivo in intracranial models. RESULTS/ANTICIPATED RESULTS: We hypothesize that WNT5A-AS1 targets Wnt5a and regulates its expression. We anticipate that knockdown of WNT5A-AS1 will upregulate WNT5A expression. We also expect that inhibiting WNT5A-AS1 will alter GSC stem maintenance and functional effects. We expect to see an increase in downstream Wnt5a signaling proteins in males vs females when treated with exogenous Wnt5a. We hypothesize that knockdown of both, WNT5A-AS1 and WNT5A will alter the expression of downstream proteins. We hypothesize that knockdown of WNT5A-AS1 will decrease tumor growth and therapeutic resistance to TMZ while increasing survival in patient derived xenographs in vivo and in vitro. DISCUSSION/SIGNIFICANCE: This study will provide insight into the mechanisms underlying the difference in GBM onset and progression between male and female patients, which is clinically important. We will also characterize the biological role WNT5A-AS1 which is currently unknown to date and elucidate differential role of GSCs in GBM progression between male and female.

429

Elevated Phosphate Levels Induce Lung Inflammation and Exacerbate Pulmonary Fibrosis*

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OBJECTIVES/GOALS: Using a cell culture model, we will determine the effects of phosphate on primary lung cell cultures and use our results to delineate a pathway through which these changes are carried out. Using animal models, we will determine the effects of phosphate on inflammatory and fibrotic lung injury, both in the presence and absence of CKD. METHODS/STUDY POPULATION: For our in vitro experiments, human lung fibroblasts were treated with concentrations of 1 to 5 mM sodium phosphate and FGFR inhibitors. Expression levels of interleukin (IL)-1beta, IL-6, and IL-8 were analyzed by qPCR and secretion of these cytokines was measured by ELISA. Phosphorylation of PLCy and ERK was measured by western blot. Using an in vivo approach, we placed C57Bl/6 mice on a high phosphate (3%) diet to elevate serum phosphate levels in the absence of kidney injury and administered bleomycin via oropharyngeal aspiration to generate an acute inflammatory response. Serum FGF23 levels were measured by ELISA and serum analysis for phosphate and renal function were obtained. Furthermore, expression of FGF23 pathway and inflammatory markers were analyzed in murine lung tissue using qPCR and western blotting. RESULTS/ANTICIPATED RESULTS: Augmented phosphate concentrations led to increased cytokine expression and secretion from human lung fibroblasts as well as a concomitant increase in PLCy and ERK phosphorylation. Inhibition of FGFR1 reversed the effects of phosphate on the inflammatory cytokines and PLCy/ERK phosphorylation. Serum FGF23 levels were significantly upregulated in mice on a high phosphate diet and further increased in mice subjected to a high phosphate diet with exposure to bleomycin. Both serum phosphate and creatinine levels were significantly elevated as well. Additionally, high phosphate and bleomycin increased local FGF23 expression in murine lung tissue, when compared to controls or each stimulus alone. DISCUSSION/SIGNIFICANCE: Phosphate has a significant impact on inflammation and fibrosis in the lung, indicating that the existence of pulmo-renal crosstalk exaggerates pulmonary injury and that there are biological pathways that may be targeted therapeutically to mediate these effects. These results could have a substantial impact on the quality of life for CKD patients.

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