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Abstract 1

Improvement in bladder parameters of 12-month-old male and female mice with THX-B treatment, an antagonist to the P75NTR receptor

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Introduction: Neuron development and survival are regulated by neurotrophins, including nerve growth factor (NGF). In female elderly people with overactive bladder syndrome (OAB), the levels of NGF in urine are reduced, which has been linked to elevated activity of the enzyme matrix metalloproteinase-9 (MMP-9), the main protease responsible for NGF degradation. These characteristics were also observed in animal models of type 1 diabetic bladder dysfunction, and improved by chronic treatment with THX-B, an antagonist of the proinflammatory receptor $p75^{NTR}$. The aim of this study was to investigate the functional benefit of $p75^{NTR}$ antagonism on bladder function in aging mice.

Methods: Male and female C57BL/6J mice, aged six and 12 months, were subjected to a four-week treatment of THX-B or PBS (control). Urination behaviors and patterns were measured using a voiding spot test. Bladder contractility was evaluated using organ baths in the presence of KCl, electrical field stimulation, and carbachol. Conscious cystometry was performed after bladder catheterization surgery to assess bladder contractility. ELISA kits were used to measure NGF and proNGF levels in urine. Immunoblotting was used to semi-quantify MMP-9, VachT, and PGP 9.5.

Results: THX-B improved voiding behavior and bladder contractility compared to the control group: total urine volume, volume per micturition, and voiding frequency were reduced in 12-month-old male and female mice. Ex-vivo, bladder contractility stimulated by KCl, electrical field stimulation, and stimulation by carbachol were reduced in strips from mice after THX-B treatment. In female mice, conscious cystometry revealed a decrease in maximal voiding pressure, basal pressure, spontaneous activity, and micturition volume by THX-B treatment compared to controls. In males, THX-B decreased the maximal voiding pressure and residual volume. Moreover, THX-B treatment increased NGF urine levels in six- and 12-month-old male and female mice, restoring the NGF/proNGF imbalance. MMP-9 activity was decreased, but only in female mice.

Conclusions: Our results suggest that the improved bladder parameters by THX-B is age-dependent and leads to improved bladder function only in 12-month-old mice. Decreased MMP-9 activity occurs exclusively in female mice, suggesting gender-specific pathways. THX-B could potentially be used as a therapeutic intervention to improve OAB in the female aging population.

Abstract 2

Renal YAP (Yes-associated protein)/TAZ (transcriptional coactivator with PDZ-binding motif) upregulation correlate with human renal fibrosis progression

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Introduction: Tubular atrophy and interstitial fibrosis lead to chronic kidney disease (CKD), which affects over 30–40 million of the U.S. population, with very limited and expensive therapies. Obstructive uropathy is one important cause of renal disease and is accentuated by diabetes and hypertension. YAP and TAZ are known nuclear transducers of the Hippo pathway, which remain inactive (low expression) during tissue homeostasis. Upregulation and/or nuclear accumulation of YAP/TAZ has been demonstrated to promote various neoplastic and fibrotic diseases. We have demonstrated, in mouse preparations of obstructive uropathy, that tubular and interstitial overexpression of YAP and TAZ are causatively linked to tubular maladaptive repair and tubulointerstitial fibrosis progression. While this has been confirmed in numerous in-vivo and in-vitro studies in various mouse CKD models, it is unclear whether YAP and TAZ expression correlate with progression of fibrosis and CKD in humans.

Methods: Renal biopsy samples from 62 patients were evaluated for degree of fibrosis using Masson Trichrome/PAS staining. TMAs constructed from patient kidney samples were evaluated for expression of YAP and TAZ by immunohistochemistry (IHC) staining. Extent (0–100%) and intensity (0–3 scale) of YAP/TAZ staining in the tubules and interstitium were blindly scored. H-score was calculated by multiplying the extent and intensity of staining. Renal tissue with moderate to severe fibrosis (>25% fibrosis, n=26) were compared to those with mild or no fibrosis (<25% fibrosis, n=36). Demographics data and medical history were collected by chart review. Two-tailed t-test was used for statistical comparison.

Results: Demographic parameters were similar across both groups; median age of the analyzed population was 51.5 and the majority of individuals identified as Caucasian (74.2%) males (59.4%). IHC analysis revealed that the >25% fibrosis group exhibited significantly higher extent of interstitial (>2.5 fold, $p<0.0001$) and tubular (>1.5 fold, $p<0.01$) YAP/TAZ staining when compared to the <25% fibrosis cohort. There was a dramatic increase in interstitial H-score (>3.5-fold, $p<0.0001$) in the >25% fibrosis group relative to the <25% fibrosis cohort.

Conclusions: In humans, renal YAP/TAZ protein expression was increased in fibrotic kidneys and the degree of increase correlated with the degree of fibrosis. This confirms that the results of our animal studies are applicable in human CKD. Therefore, YAP and TAZ could be novel renal tissue biomarkers of CKD progression and potential therapeutic targets.

Abstract 3

Design and development of specific protein phosphatase-5 inhibitors for the treatment of VHL-null clear-cell renal cell carcinoma

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Introduction: Despite good survival rates for localized renal cell carcinoma a proportion of patients present with or will progress to metastatic disease, which portends worse prognosis and poor overall survival. Although survival has been improved with the use of new targeted therapies and immunotherapy regimens, many patients will ultimately develop resistance. There is therefore a need for identification and development of new therapeutics. Clear-cell renal cell carcinoma (ccRCC), the most common histologic subtype, generally harbors loss of the tumor suppressor von Hippel Lindau (VHL). The serine/threonine protein phosphatase-5 (PP5) plays a role in the regulation of numerous signaling pathways essential for cancer growth. Previously, we have shown that PP5 is targeted for ubiquitination and degradation by a VHL-containing E3 ubiquitin ligase complex in a hypoxia-independent manner. Expression and activity of PP5 is consequently increased in ccRCC, and it plays a pro-survival role. Knock-down of PP5 expression or targeting its activity through inhibition of the kinase casein kinase-1δ (CK1δ) caused apoptosis in VHL-null ccRCC. The objective of this study was to develop a novel PP5 inhibitor for ccRCC.

Methods: An in-silico docking screen using the crystal structure of the PP5 active site and a large compound library was used to identify candidate inhibitors. A series of candidates were then evaluated using both in-vitro and cell-based assays for their ability to inhibit PP5 activity and cause apoptosis in ccRCC cells. In-vitro PP5 activity was assessed using specific phospho-peptide substrates and in cells by immunoblotting to assess phosphorylation levels of known substrates. Two primary candidates were conjugated to biotin for PP5 pulldown experiments to assess specificity.
Results: Our in-silico screen identified approximately 200 candidate inhibitors. Further screening of about 20 of these compounds identified two compounds, P5 and P13, which inhibit PP5 activity both in-vitro and in ccRCC cells and were further refined based on structure. Pulldown using biotin-conjugated drugs demonstrated good inhibitor specificity. Furthermore, treatment with these inhibitors leads to apoptosis in VHL-null ccRCC cell lines.
Conclusions: PP5 promotes cell survival in ccRCC and knockdown or inhibition of PP5 leads to cellular apoptosis. We have identified a small molecule which specifically binds to and inhibits PP5 catalytic activity. This novel PP5 inhibitor causes apoptosis in VHL-null ccRCC cells and may serve as a new therapeutic strategy for treatment of advanced ccRCC.
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Abstract 4

Diabetic bladder dysfunction: Characterization in tally ho mice, a diabetic type 2 mouse model

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Introduction: Diabetes mellitus is a prevalent metabolic disease affecting multiple organ systems, including diabetic bladder dysfunction (DBD). Animal models provide a better understanding of the pathophysiology behind this complex disease. We aimed to characterize the bladder function of a diabetic type 2 mouse model, Tally Ho, as it progresses from an early overactive to a decompensated underactive state.
Methods: Tally Ho (TH) mice were used as our diabetic type 2 mouse model whereas SWR/J mice were used as control mice. Six mice per strain were studied at various time intervals (10, 14, and 18 weeks of age). General characteristics such as body weight, glycemia and bladder weight were collected. Voiding spot assays over a four-hour timeframe looked at total urine volume, number of voids, and volume per void. Suprapubic catheters were implanted for urodynamic studies measuring voiding pressure, intermicturition pressure, bladder capacity, bladder compliance, postvoid residual volume, intermicturition time interval, and voided volume. Detrusor smooth muscle strips were stimulated using KCl 60 mM, electrical field stimulation (1, 2, 4, 8, 16, 32 Hz) and carbachol (from 3 nM to 100μM) to assess contractility.
Results: Our results show that TH mice had a significantly larger body weight than SWR/J mice at 10 weeks of age and this persisted at 18 weeks of age. There was no difference in glycemia and bladder weight at 10 weeks of age, however, by 18 weeks, the male TH had significantly higher glycemia and all TH mice had significantly larger bladder weights when compared to SWR/J mice. At 10 weeks of age, the TH mice voided larger total volumes and more frequently compared to their SWR/J counterparts, however, the volume per void was reduced. This shifts at 18 weeks when the TH mice void per volume becomes significantly larger than the SWR/J mice. TH mice had significantly larger bladder capacities and postvoid residuals compared to SWR/J mice at 18 weeks of age. At 10 weeks, there was no difference in detrusor smooth muscle contractility in response to KCl, EFS and carbachol, however, at 18 weeks, TH mice had consistently higher detrusor smooth muscle contractility to KCl, EFS, and carbachol compared to the SWR/J mice.
Conclusions: Tally Ho mice can be used as a diabetic type 2 mouse model for DBD. This mouse model demonstrates the change in bladder morphology as diabetes progresses. This characterization opens the door for future studies to identify specific targets and therapies in DBD.

Abstract 5

Uremic toxins directly promote calcium oxalate crystallization and enhance stone adherence by inducing oxidative stress

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Introduction: Approximately one in 10 people will develop a kidney stone in their lifetime, with most stones containing either calcium oxalate (CaOx, ~80%) or calcium phosphate (~20%) as the primary constituent. The inadequacies in our understanding of the disease etiology are reflected by the increasing prevalence of renal calculi and must be addressed. The mechanisms behind vascular and renal calcification are highly similar. Interestingly, the gut microbiota of stone formers is primed with bacterial species that produce uremic toxins associated with vascular calcification. The uremic toxins indoxyl sulfate, p-cresyl sulfate, and their precursors enhance the production of reactive oxygen species (ROS) in the kidneys and can cause calcium concentrations to increase in the blood of humans. By having increased access to calcium and experiencing oxidative stress, renal cells damaged by uremic toxins may act as crystal nucleation sites for kidney stone production. We have previously shown that uremic toxins increase stone burden in-vivo, therefore, we sought to determine how uremic toxins and their precursors enhance CaOx stone formation using both in-vitro and in-vivo models.
Methods: The direct effect of uremic toxins on CaOx crystallization was assessed in a simulated urine environment and with a standard gel-based assay. Mammalian kidney cells were exposed to uremic toxins to determine if they alter CaOx adherence. A Drosophila melanogaster model was used to investigate if the uremic toxins enhance stone burden in-vivo by inducing oxidative stress or through gut microbiota changes.
Results: Indoxyl sulfate, p-cresyl sulfate, and their precursors directly promoted CaOx crystallizationin artificial urine and the gel-based assay. Uremic toxin exposure significantly reduced mammalian kidney cell viability and enhanced CaOx adherence. Stone burden in D. melanogaster Malpighian tubules (i.e., fly kidney) had a strong and positive correlation with ROS production. Uremic toxin exposure reduced the abundance lactobacilli in the Drosophila gut microbiota, which is common in human stone formers.
Conclusions: This is the first study to show that uremic toxins directly promote CaOx crystallization in urine and gel-based assays. These uremic toxins enhance stone burden in-vivo by promoting ROS production in the renal environment. Together, these data suggest uremic toxins may bolster the production and adherence of stones to kidney cells, which we verified using cell culture. This work will be of particular interest to clinicians for the development of novel therapeutic and preventative measures that target uremic toxin accumulation to prevent kidney stone disease.