RNA Sequencing of Renal Tubular Epithelial Cells Uncovers Novel Players in Renal Fibrosis

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Background: Tubular epithelial cells (TECs) play an important role in the development of renal fibrosis. When injured, they undergo dedifferentiation. Dedifferentiated TECs secrete cytokines that promote transdifferentiation of neighboring pericytes and fibroblasts into extracellular matrix (ECM)-producing myofibroblasts. TGF-β1 is a key fibrogenic cytokine that promotes TEC dedifferentiation after renal injury. Therapies targeting TGF-β1, however, have not been successful for treatment of renal fibrosis. This is likely because TGF-β1 also has protective functions, including anti-inflammatory effects and regulation of autophagy. Here, we used a transcriptomic approach to identify molecules downstream of TGF-β1 that specifically execute its pro-fibrotic functions.

Methods: Cultured human proximal tubular epithelial cells (HKT) were treated with either 2.5 ng/mL of TGF-β1 or vehicle for 72 hours. Their RNA was extracted and subjected to RNA sequencing. Transcriptomic data from the renal tubulointerstitium of patients with various forms of chronic kidney disease (CKD) was also obtained via the GEO database. Genes significantly upregulated at the mRNA level in HKT after TGF-β1 treatment were compared to transcriptomic data from diseased tubulointerstitium. The genes that were upregulated in diseased tubulointerstitium across 3 or more GEO datasets and in TGF-β1-treated HKT were chosen for further evaluation.

Results: MARKS and DOCK2 were the 2 genes from our in vitro RNA sequencing data that were most consistently and most highly upregulated across multiple GEO datasets. Their upregulation in HKT was confirmed by qPCR and western blot. Next, HKT were transfected separately with siRNA against each gene and then treated with TGF-β1. Knocking down MARKS prevented upregulation of COL1A1 and cSMA by TGF-β1 in HKT. However, knocking down DOCK2 further increased the upregulation of cSMA by TGF-β1 in HKT.

Conclusions: MARKS is likely a mediator of the fibrogenic activity of TGF-β1, given that knocking it down ameliorates the TGF-β1-induced fibrogenic changes in HKT. DOCK2 may be a negative regulator of TGF-β1 activity, upregulated as part of a negative feedback response. The functions of these molecules in tubular epithelial cells in the context of renal fibrosis need to be further characterized in an animal model of chronic kidney injury.

Funding: Private Foundation Support

Adenine Stimulates mTORC1 to Increase Matrix Protein Synthesis, Which Is Inhibited by Hydrogen Sulfide in Kidney Proximal Tubular Epithelial Cells

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Background: Adenine promotes chronic kidney disease (CKD) and cardiovascular damage in rodent models. However, the underlying mechanism is poorly understood. Hydrogen sulfide (H2S) deficiency is associated with kidney injury seen in aging and CKD which is ameliorated by exogenous H2S supplementation. We hypothesize that adenine promotes kidney injury by activating mTORC1 to increase matrix protein synthesis which is ameliorated by H2S.

Methods: We employed mouse kidney proximal tubule epithelial (MCT) cells. LY294002, MK2206 and rapamycin were used as inhibitors of PI3K, Akt and mTORC1, respectively. Sodium hydrosulfide (NaHS) was used as a source of H2S.

Results: Adenine increased S6K phosphorylation, an index of mTORC1 activity, and fibronectin expression in a dose- and time-dependent manner. Inhibitors of PI3K, Akt and mTORC1 abolished adenine-induced S6K phosphorylation. mTORC1 inhibitor also ameliorates adenine-induced fibronectin expression. Administration of NaHS inhibited adenine-induced S6K phosphorylation to ameliorate fibronectin accumulation.

Conclusions: Our results indicate that adenine-stimulated mTORC1 is regulated by PI3K-Akt signaling. PI3K-Akt-mTORC1 axis mediates adenine-induced matrix protein synthesis which is ameliorated by exogenous H2S administration. Inhibition of mTORC1 by H2S could be used as a therapeutic intervention for adenine-induced CKD.

Effects of Sacubitril/Valsartan on Hypertension in Patients With CKD Stage 5D

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Background: The purpose of this study was to evaluate the efficacy and safety of Sacubitril/Valsartan (SV) compared with angiotensin receptor blocker (ARB) in dialysis-dependent patients with CKD stage 5D (CKD stage 5D) complicated with hypertension, and to provide a reliable basis for the treating hypertension in patients with CKD stage 5D. The preliminary results of this study suggested that SV safely and effectively reduce blood pressure in patients with CKD stage 5D, and might be superior to ARB. The study also looked at the effects of SV on survival benefit, residual kidney function and blood pressure, and results will be released in due course.

Table 1. SBP was lower in the SV group than in the ARB group at 3 and 9 months (P = 0.041,0.005) (Table 1). During the follow-up period, SV was safe and well-tolerated in dialysis patients during.

Results: 452 patients with CKD stage 5D (268 of HD and 254 of PD) were enrolled, with a median age of 58 (23-75) years old, and the male to female ratio was 1.9 to 1. 263 patients in SV group and 259 patients in ARB group. The 1 year treatment with SV 100–200 mg per day resulted in significantly reductions in mean BP from baseline(Figure 1). The mean sitting systolic BP (mSBP) was reduced from 149 ± 20 mm Hg at baseline to 144 ± 18 mm Hg, 141±32 mm Hg, 137±17 mm Hg, 140±19 mm Hg at 3, 6, 9 and 12 months (P<0.03, 0.02, <0.001, 0.004). The mSBP was lower in the SV group than in the ARB group at 3 and 9 months (P = 0.041,0.005) (Table 1). During the follow-up period, SV was safe and well-tolerated in dialysis patients during.

Conclusions: The preliminary results of this study suggested that SV safely and effectively reduce blood pressure in patients with CKD stage 5D, and might be superior to ARB. The study also looked at the effects of SV on survival benefit, residual kidney function and blood pressure, and results will be released in due course.

Development and Validation of Multivariable Prediction Models of Serological Response to SARS-CoV-2 Vaccination in Kidney Transplant Recipients

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Background: Repeated vaccination against SARS-CoV-2 increases serological response in kidney transplant recipients (KTR) with high interindividual variability. Still, no decision support tool exists to predict SARS-CoV-2 vaccination response in KTR.

Methods: We developed, internally and externally validated five different multivariable prediction models of serological response after the third and fourth vaccine dose against SARS-CoV-2 in KTR. Using 27 candidate predictor variables, we applied statistical and machine learning approaches including logistic regression (LR), LASSO LR, random forest, and gradient boosted regression trees. For development and internal validation, data from 585 vaccinations were used. External validation was performed in four independent, international validation datasets comprising 191, 184, 254, and 321 vaccinations, respectively.

Results: Internal validation using a rigorous resampling approach showed AUC-ROC of 0.825 for LASSO LR, which was then used for model fitting and external validation. LASSO LR performed on the whole development dataset yielded a 23- and 11-variable model, respectively. External validation showed ROC-AUC of 0.855, 0.749, 0.828, and 0.763 for the sparser 23-variable model, yielding an overall AUC-ROC of 0.809, and a negative predictive value of 0.752. The 23-variable model showed AUC-ROC of 0.853, 0.714, 0.844, and 0.778 in four independent validation sets, yielding an overall AUC-ROC of 0.818, and a negative predictive value of 0.795.

Conclusions: Both, an 11- and 23-variable LASSO LR model predict vaccination response in KTR with good AUC-ROC. Implemented as an online tool at https://www.tx-vaccine.com, it can guide decisions when choosing between different immunization strategies to improve protection against COVID-19 in KTR.
Inhibition of Toll-Like Receptor 7 (TLR7) With a Selective Inhibitor of Human TLR7 ST-301 Reverse Lupus Progression in Murine Lupus Models

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Background: Toll-like receptor 7 (TLR7) is an endosomal innate viral RNA sensor, primarily expressed in plasmacytoid dendritic cells and B cells. A large body of evidence supports enhanced TLR7 signaling as a mechanism of human systemic autoimmune disease. Blocking the TLR7 pathway has been investigated in SLE disease models. ST-301 is a potent selective TLR7 inhibitor and has similar human and mouse potency. ST-301 was used to investigate the role of TLR7 signaling in murine lupus models.

Methods: MRL/lpr and NZB/W mice were treated with a vehicle or a selected dose of ST-301, prednisolone, and ST-301 plus prednisone. MRL/lpr mice were treated for 6 weeks on the early-stage disease with positive anti-dsDNA antibodies but negative proteinuria. NZB/W mice were treated for 20 weeks on the early-stage disease with positive anti-dsDNA antibodies but negative proteinuria, twelve weeks on established disease (proteinuria <100 mg/dL), and eight weeks on advanced disease (proteinuria >100 mg/dL).

Results: Six weeks of treatment with ST-301 on the MRL/lpr early-disease mice provided a significant delay of proteinuria onset, reduction of autoantibody production, and inhibition of IgG deposition in the kidney. Twenty weeks of treatment with ST-301 on the NZB/W early-disease mice resulted in a significant delay in the onset of lupus nephritis, onset of proteinuria, reduction of serum IgG level, and IgG deposition in the kidney. Treatment of NZB/W mice with ST-301 on established disease mice for twelve weeks and advanced disease mice for eight weeks resulted in a reduction of proteinuria, kidney IgG deposition, interstitial fibrosis, and a significant increase in survival in both established and advanced disease mice models. Serum autoantibody levels were significantly reduced in early-stage, established, and advanced disease mice models.

Conclusions: The novel highly selective TLR7 inhibitor ST-301 displayed robust efficacy in delaying the onset of Lupus nephritis, progression of Lupus nephritis, and survival benefit on MRL/lpr and NZB/W Murine Lupus Models. ST-301 significantly reduces proteinuria, autoantibodies production, and immune complex deposition. Resulting in a significant decrease of kidney interstitial fibrosis and other disease models. These results support that inhibiting the TLR7 pathway is a potential treatment for SLE.

Funding: Commercial Support - Singularity Therapeutics INC, Hillhouse Medical Group PLLC, Private Foundation Support.