POSTER SESSION: CHRONIC KIDNEY DISEASE, EXPERIMENTAL MODELS, BIOMARKERS, PRECISION MEDICINE

POS13
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POS-358

SERUM NITRIC OXIDE AND NOT ASYMMETRIC DIMETHYL ARGININE OR MATRIX METALLOPROTEINASE 2 DETECTS ENDOTHELIAL DYSFUNCTION IN AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE PATIENTS WITH EARLY DISEASE

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Introduction: Cardiovascular complications are the leading cause of death in patients with autosomal dominant polycystic kidney disease (ADPKD) and there is a high incidence of hypertension in ADPKD patients with early disease. Endothelial dysfunction (ED) is an early marker of cardiovascular complications and this has also been reported in ADPKD. However, published studies have included a significant number of patients with later stage disease and other known comorbidities that are also associated with ED including hypertension. The major aim of this work was therefore to test the hypothesis that ED was present in ADPKD patients with early disease

Methods: A cross-sectional study was first performed using retrospective serumsamples obtained from 60 ADPKD patients, 41 patients with other forms of chronic kidney disease (OCKD) and 36 healthy volunteers. In a subsequent prospective study, 20 ADPKD patients with eGFR ≥60ml/min attending the PKD clinic at the Sheffield Kidney institute were recruited. Exclusion criteria included hypertension, current smokers, age above 50 years, high body mass index ($>30kg/m^2$) and concurrent use of medications known to be associated with ED. A total of 20 age, sex and eGFR matched healthy volunteers were also recruited. Endothelial function was assessed non-invasively by reactive hyperemic index (RHI). Serum concentrations of asymmetric dimethyl arginine (ADMA), matrix metalloproteinase 2 (MMP2), and nitrite/nitrate were measured as markers of ED. Blood was collected for routine biochemistry and urine for 24hr protein and creatinine measurements.

Results: In the initial retrospective cross sectional study, marker of endothelial dysfunction (serum ADMA) was significantly higher in ADPKD patients than in HV but were similarly elevated in patients with OCKD. In the prospective study, there was a significant difference in diastolic blood pressure (DBP) and proteinuria between PKD and HV groups despite the absence of diagnosed hypertension or difference in eGFR ($p<0.001$) RHI was not significantly different between PKD patients and controls. Similarly, no difference in ADMA and MMP2 were found. Conversely, serum nitrite/nitrate was lower in ADPKD patients than in controls.

Conclusions: Although baseline endothelial function was normal as measured by RHI and some serum biomarkers in ADPKD patients with early disease (eGFR>60), the presence of increased proteinuria, lowered serum nitrite/nitrate concentrations correlating with higher DBP suggest some features of ED may be present in early PKD. The detection of similar increases in ADMA and 8-isoprostane in ADPKD with late disease (eGFR<60ml/min) and OCKD suggests similar mechanisms for ED in both groups rather than a PKD specific pathway of ED.

No conflict of interest

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DEVELOPING TARGETED THERAPIES FOR KIDNEY DISEASE IN PUBLIC PRIVATE PARTNERSHIPS

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Introduction: Integrating public and private research efforts is key to expediting precision medicine for glomerular diseases. The Nephrotic Syndrome Study Network (NEPTUNE) has developed an innovative research and business model: "MATCH", to enable targeted patient enrollment in clinical trials via the NEPTUNE public private partnership (NP5).

Methods: The NEPTUNE community knowledge base is leveraged to identify a molecular signal matching therapeutic targets under development by NP5 industry partners. The three steps of MATCH are: (1) predictive biomarker development; (2)patient communication about precision medicine; and (3) return of results from NP5 partner clinical trials for evaluation of patient stratification effects on trial outcomes.

Results: MATCH begins with NEPTUNE patient education on risks and benefits of the study. Once enrolled in MATCH, a patient's biosamples undergo biomarker profiling. MATCH provides individual pathway activation information to patients for consideration in their independent decision to enroll in a trial that may be more tailored to their specific disease and stage. Finally, clinical trial outcomes will be reported by NP5 partners to NEPTUNE to evaluate the MATCH impact on treatment responses.

Conclusions: MATCH aims to assess the ability to communicate molecular information to patients, and to test precision medicine across multiple trials and partners to move science closer to the overall goal: reaching the right patient with the right medicine at the right time. MATCH is an open framework available to additional partners interested in joining NP5.

No conflict of interest

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THE ROLE OF PHOTOBIOMODULATION ON CHRONIC KIDNEY DISEASE

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Introduction: Chronic kidney disease (CKD) is a worldwide public health problem, with adverse outcomes of kidney failure, cardiovascular disease and premature death. Accumulating studies have suggested that inflammation, mitochondrial dysfunction, and oxidative stress play pivotal roles in the development and progression of CKD. The implementation of evidence-based clinical practices only slows its progression to end-stage kidney disease (ESKD). Kidney replacement therapy (transplantation or dialysis) and death are the consequences of ESKD, resulting in a significant burden on the health system. Hence, there's an urgent need for novel therapies for CKD. Photobiomodulation (PBM), the use of non-thermal light for therapeutic purposes, has been used clinically for more than 50 years to treat a wide range of diseases. PBM is effective in mitigating inflammation, mitochondrial dysfunction, and oxidative stress, all of which are factors inherent in CKD. However, the potential role of PBM in treating CKD has not been explored. This study aims to identify the direct effects of PBM in *in vitro* model of CKD.

Methods: In vitro human proximal tubular cells (HK2 cells) were pretreated with low ($1.38\ J/cm^2$) or high dosage ($2.75J/cm^2$) of PBM. 24 hours after pretreatment, cells were incubated with TGF-β1 (2 ng/ml) for 48 hours with or without PBM irradiation every 24h. The SHAM groups were processed under the exact same conditions except in the absence of PBM irradiation. The mRNA expression of the inflammatory markers monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-) and fibrotic marker fibronectin were measured by qRT-PCR.

Results: TGF-β1 significantly increased the mRNA expression of MCP-1 (3.5 fold, $P<0.001$) and TNF-α (2.1 fold, $P<0.01$) in HK2 cells when compared to control group. Irradiation of HK2 cells with PBM at a dose of $1.38\ J/cm^2$significantly reversed TGF-β1-induced upregulation of MCP-1 ($P<0.001$) and TNF-α expression ($P<0.001$), when compared with the SHAM group. However, PBM at the dose of $2.75J/cm^2$did not change the expression of MCP-1 and TNF-α compared with the SHAM

group. Moreover, the mRNA expression of fibronectin was significantly upregulated in TGF-β1-stimulated HK2 cells (2.1 fold, $P < 0.001$) when compared to control group. The application of PBM at the dose of 1.38 J/cm^{2}significantly inhibited TGF-β1-induced overexpression of fibronection ($P < 0.05$) in HK2 cells when compared with the SHAM group. There was no effect of PBM at the dose of 2.75J/cm^{2}on the fibronectin expression in HK2 cells.

Conclusions: The present study suggests that PBM directly reduced inflammation and fibrosis in kidney tubular cells, when used at the appropriate dose.

No conflict of interest

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β-CATENIN-CONTROLLED TUBULAR CELL-DERIVED EXOSOMES PLAY A KEY ROLE IN FIBROBLAST ACTIVATION VIA THE OPN-CD44 AXIS SIGNALING

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Introduction: Chronic kidney disease (CKD) is a high-risk factor of end-stage renal disease (ESRD). However, the therapeutic strategies for CKD remain unsatisfactory.

Exosome-mediated tubule-interstitial communication was recently shown to be a main mechanism underlying the development of renal fibrosis. Nevertheless, the detailed mechanisms remain to be elucidated.

Osteopontin (OPN) is an extracellular matrix glyco-phosphoprotein, which was first identified in bone tissue and then found to be expressed in other tissues, such as kidney, teeth, and blood. In the diseased state, full length OPN was often cleaved into an N-terminal fragment (~50 kDa) and two C-terminal fragments (~18 kDa and ~16 kDa). N-OPN could be the major active form to trigger a series of pathological changes. However, the role of N-OPN in kidney injury has not yet been investigated. Furthermore, the underlying mechanisms and modulating signals are poorly understood.

Methods: We examined the expression of N-OPN in various clinical nephropathies and quantitatively measured the levels of N-OPN in 30 healthy subjects and a cohort of 183 patients with CKD using a specific ELISA. We then isolated exosomes from the urine of CKD patients and tested its protein constitution by SDS PAGE with Coomassie Blue staining and mass spectrometry.

In mouse models of ischemia-reperfusioninjury (UIRI) and unilateral ureteral obstruction (UUO), we performed experiments by intravenous injection of OPN expression vector (pCMV-OPN) and concomitantly treated mice with DMA to block the release of exosomes. Renal function and multiple fibrogenesis-related proteins were examined. To clarify the mechanism, gene deletion of β-catenin in tubular cells (Ksp-β-catenin−/−) or gene ablation of CD44 (CD44−/−) were constructed.

HKC-8 cells were transfected with pDel-β-catenin, followed by incubating in serum-free medium. In some experiments, HKC-8 cells were pretreated with DMA or co-transfected with OPN siRNA. NRK-49F cells were treated with conditioned media from HKC-8 cells or exosomes (pcDNA3-Exo or active β-cat-Exo) isolated from HKC-8 cells transfected with or without pDel-β-catenin. In some experiments, NRK-49F cells were pretreated with human recombinant OPN or transfected with CD44 siRNA. Multiple fibrogenesis-related or proliferation-related proteins were examined by western blot analysis or immunofluorescence staining.

Results: OPN, especially N-OPN, was increased in injured tubules and secreted into the urine by exosomal capsulation. Urinary N-OPN served as an indicative marker for renal fibrosis and decline in kidney function. OPN-encapsulated exosomes mediate intercellular message transmission from tubular cell injury to interstitial fibrosis, through the receptor of CD44 in fibroblasts. We further identified that OPN is a new downstream target of β-catenin, a master controller in tubular injury and fibrogenesis. As a result, β-catenin is a central regulator of the exosome-mediated OPN/CD44 axis in renal fibrosis.

Conclusions: These studies showed that exosomes play an important role in promoting renal fibrosis by mediating communication between tubular epithelial cells and fibroblasts. Moreover, a large number of exosomes are induced in tubular epithelial cells after kidney injury, with contents including OPN and N-OPN. Finally, exosome-mediated OPN/CD44 axis activation is controlled by β-catenin signaling.

No conflict of interest

POS-362

DEVELOPMENT OF BIOMARKERS FOR PREDICTING DKD PROGRESSION IN NON-PROTEINURIC TYPE 2 DIABETES: THE NTUH-DKD COHORT STUDY

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Introduction: Diabetic kidney disease (DKD) is one of the leading causes of chronic kidney disease (CKD) and end-stage renal disease (ESRD) worldwide. Despite being a diagnostic criterion for DKD, the predictive value of urine albumin-creatinine ratio (UACR) on the progression toward advanced CKD is limited, due to the heterogeneous disease nature. Better predictive biomarkers are urgent needed to help us to better manage DKD patients. In this prospective cohort study, we evaluated the potential of incorporating a novel DKD Biomarker, a post-Translational Modification (PTM) fragment of Fetuin-A, into clinical parameters for predicting short-term DKD Progression in non-proteinuric type 2 diabetes (T2DM).

Methods: 308 patients with T2DM and UACR < 300 mg/g were enrolled in this study. At recruitment and every 6 months, clinical measurements and a urine sample were collected. Removing 18 withdraws, 290 individuals were included for the study. The concentration of a PTM Fetuin-A fragment was measured with BPM's DNlite-IVD103 test, and adjusted (values were log10 transformed after divided by urine creatinine to approximate normality, denoted by IVD103 hereafter) to evaluate.

The definition of DKD progression was the composite of incident DKD (estimated glomerular filtration rate [eGFR] <60 mL/min/1.73 m^2) and ≥30% eGFR decline for individuals with baseline eGFR above and below 60 mL/min/1.73 m^2. To evaluate short-term risk, 2-year follow-up time was adopted and eGFR was calculated with the CKD-EPI equation.

Multiple logistic regression approach was used to investigate 3 common used clinical parameters (eGFR, HbA1c, and UACR) of DKD Progression (referred to as "clinical model"). The "clinical plus IVD103 model" is obtained by adding IVD103 into the clinical model. Model discrimination was assessed by the area under the curve (AUC). The Youden index was used to determine the optimal cut-off in each model. Improvement in AUC and the expected information for discrimination (Λ) were presented.

To make a convenient validation, the NTUH-DKD cohort truncated at Visit 5 (2 year from baseline) was treated as "validation cohort". The algorithm obtained from the development cohort (2 years from baseline) was applied to validation cohort and the performance were assessed.

Results: During 2 years follow-up, DKD Progression developed in 18 individuals (6.2%). In the clinical model, baseline eGFR and HbA1c are significant predictors but not UACR. In clinical plus IVD103 model, higher IVD103 is also a significant predictor (Odds ratio=2.98, p-value=0.033). IVD103 improved model fit (ΔLRT=4.92, p-value=0.027), discrimination (AUC increase from 0.85 to 0.89, p-value=0.071), sensitivity and negative predictive value (increased from 83.3% to 88.9% and from 98.6% to 99.1%, respectively), and the expected information for discrimination (Λ increased from 1.20 to 1.48).

In the validation cohort, DKD Progression developed in 27 individuals (9.3%). The developed algorithm provided good discrimination (AUC=0.81) and at the optimal cut-off provided 81.5% sensitivity and 97.3% negative predictive value to predict 2-year risk of DKD Progression.

Conclusions: In this prospective cohort study, the combination of available clinical parameters and IVD103 produced an accurate prognostic test for short-term DKD progression in T2DM. Moreover, IVD103, a novel biomarker, plays a significant role in the precision management of DKD.

Conflict of interest

Potential conflict of interest:

Dr. Huang and Dr. Tseng from Bio Preventive Medicine Corp.(BPM) have shares of BPM.