MO286 MIR-155 DEFICIENCY PROTECTS RENAL TUBULAR EPITHELIAL CELLS FROM TELOMERES AND GENOMIC DNA DAMAGE IN CISPLATIN-INDUCED ACUTE KIDNEY INJURY

Qing Yin[1], Bin Wang[2] and Bicheng Liu[2]

[1]Southeast University School of Medicine, Nanjing, P.R. China and [2]Zhong Da Hospital, Southeast University School of Medicine, Nanjing, P.R. China

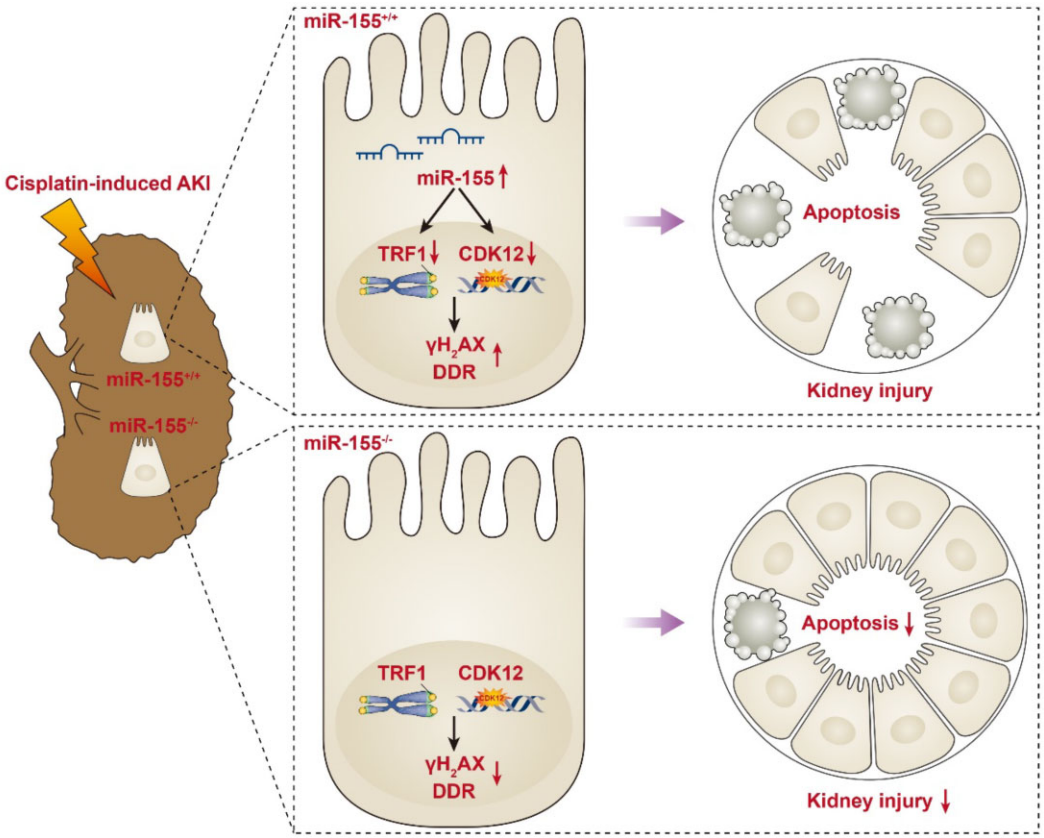
BACKGROUND AND AIMS: Cisplatin nephrotoxicity is an important cause of acute kidney injury (AKI), limiting cisplatin application in cancer therapy. Growing evidence had suggested that genome instability, telomere dysfunction and DNA damage were involved in the tubular epithelial cells (TECs) damage in cisplatin-induced AKI (cAKI). However, the exact mechanism is largely unknown.
METHOD: We subjected miR-155$^{-/-}$ mice and wild-type controls, as well as human proximal tubule cells, to cisplatin-induced AKI models. We assessed kidney function and injury with standard techniques and measured telomere by the fluorescence *in situ* hybridization.
RESULTS: Our study demonstrated that miR-155 deficiency significantly improved renal function and attenuated pathological damage and mortality during cAKI. Furthermore, miR-155 inhibition reduced cisplatin-induced TECs apoptosis, genome instability, telomere dysfunction and DNA damage both *in vivo* and *in vitro*. The miR-155 inhibition mediated protective effects may be caused by increasing expression of telomere repeat binding factor 1 (TRF1) and cyclin-dependent kinase 12 (CDK12), thereby limiting the telomere dysfunction and the genome DNA damage in cAKI.
CONCLUSION: We demonstrated that inhibition of miR-155 ameliorates AKI involving the targeting and regulation of TRF1 and CDK12, indicating a novel regulatory mechanism and elucidating a potential target for cisplatin-induced AKI treatment.

MO287 A RECOMBINANT BIO-HDL (CER-001) CAN PREVENT SARS-COV2-INDUCED RENAL DYSFUNCTION BY RESTORING SR-BI SIGNALLING

Rossana Franzin[1,1], Alessandra Stasi[1], Marco Fiorentino[1], Fabio Sallustio[1,2], Ronald Barbaras[3], Connie Peyrottes[3], Cyrille Tupin[3], Giuseppe Castellano[4], Paola Pontrelli[1] and Loreto Gesualdo[1]

[1]Nephrology Unit, Department of Emergency and Organ Tranplantation, University of Bari, BARI, Italy, [2]Department of Interdisciplinary Medicine, University of Bari, BARI, Italy, [3]Abionyx Pharma, France and [4]Department of Clinical Sciences and Community Health, University of Milan, University of Milan, Italy

BACKGROUND AND AIMS: Replication of the enveloped SARS-COV2 virus can alter lipidomic composition and metabolism of infected cells [1]. These alterations commonly result in a decline in HDL, total cholesterol and LDL, and an increase in triglyceride levels in COVID-19 patients. Furthermore, the 'cytokine storm' subsequent to release of inflammatory cytokines can severely impair lipid homeostasis. Importantly, decreased HDL-cholesterol correlates with severity of COVID-19 infection and represents a significant prognostic factor in predicting poor clinical outcomes [2]. Similarly, it has been observed that COVID-19 patients' recovery is accompanied by a rise in serum HDL levels. Pharmacological intervention that aims to restore ApoA-1 or functional HDL particles may have beneficial roles for clinical outcome of COVID-19 patients and has recently been approved for compassionate use [3].
SARS-CoV 2 spike proteins S1 and S2 can bind free cholesterol and HDL-bound cholesterol, facilitating virus entry by binding the ACE2 co-receptor Scavenger Receptor-BI (SR-BI) [4]. When activated at the trans-membrane level, SR-BI signalling culminates in Ser1173-eNOS phosphorylation with both anti-inflammatory and anti-apoptotic effect. We hypothesized that SARS-COV2 binding promoted SR-BI internalization, so that it could not exert its essential protective function. Therefore, the aim of this study is to evaluate the effects of CER-001, a mimetic HDL, in antagonizing this process.
METHOD: Endothelial and tubular (RPTEC) cells were exposed to S1, S2 and S1 + S2 (50–250 nM) with or without CER-001 (CER-001 50–500 ug/mL) and cholesterol (10–50 uM). Apoptosis tests (MTT and AnnV/PI) were performed. Internalization of SR-BI, ACE2 with S1 and activation of eNOS was evaluated by FACS analysis. SR-BI and ACE2 expression were evaluated on kidney biopsies from COVID-19 patients.



RESULTS: At concentrations used, the exposition of S1, S2 and S1 + S2 in the presence of CER-001 and cholesterol did not induce apoptosis of endothelial cells and RPTEC. Endothelial and tubular cells stimulated by S1, in presence of cholesterol, showed an increased intracellular level of SR-BI and ACE-2, with significantly reduced eNOS phosphorylation compared to baseline ($P < 0.05$). The treatment with CER-001 reversed trans-membrane SR-BI levels and eNOS phosphorylation to baseline values. The detection of S1 spike protein by endothelial cells immunohistochemistry revealed an increased level in S1-exposed cells with cholesterol and reduced S1 intracellular positive staining in CER-001-exposed cells ($P < 0.05$). Interestingly, S1-exposed cells without cholesterol appeared not to be capable of mediating S1 spike protein internalization.
Consistent with *in vitro* results, analysis of renal biopsies from COVID-19 patients with proteinuria showed increased SR-BI and ACE-2 cytoplasmic signals and reduced expression at the apical domain of injured tubules.
CONCLUSION: Our data confirmed the key role of lipid profile in SARS-COV2 infection, evaluating the molecular signalling involved in HDL metabolism and inflammatory processes, and could offer new therapeutic strategies for COVID-19 patients.

REFERENCES

1. Yan B, Chu H, Yang D *et al*. Characterization of the lipidomic profile of human coronavirus-infected cells: implications for lipid metabolism remodeling upon coronavirus replication. *Viruses* 2019; 11 (1): 73.
2. Hu X, Chen D, Wu L et al. Declined serum high density lipoprotein cholesterol is associated with the severity of COVID-19 infection. *Clin Chim Acta* 2020; 510: 105–110.
3. https://www.abionyx.com/images/pdfs/cp/2021/ABIONYX_PR_01032022_AAC_EN_c5c7d.pdf.
4. Stasi A, Franzin R, Fiorentino M *et al*. Multifaced roles of HDL in sepsis and SARS-CoV-2 infection: renal implications. *Int J Mol Sci* 2021; 22: 5980.

MO288 KIDNEY TUBULE POLYPLOIDIZATION PRESERVES RESIDUAL KIDNEY FUNCTION AND ASSURES SURVIVAL DURING ACUTE KIDNEY INJURY

Letizia De Chiara[1], Elena Lazzeri[1] and Paola Romagnani[1,2]

[1]Department of Experimental and Clinical Biomedical Sciences, Università degli studi di Firenze, Firenze, Italy and [2]University Hospital Meyer, Firenze, Italy

BACKGROUND AND AIMS: Acute Kidney Injury (AKI) is characterized by a rapid deterioration of kidney function. Recently, we showed that tubular epithelial cells (TC) respond to AKI by triggering polyploidy, a condition in which a normally diploid cell acquires additional sets of chromosomes. Polyploidy offers several advantages, but in the kidney the biological significance of polyploidization remains unclear. In this study we hypothesized that polyploidy (i) is the predominant cellular response early during AKI and (ii) that is an adaptive stress response required to maintain a residual kidney function assuring survival.
METHOD: To address these hypotheses, we employed *in vivo* transgenic models based on the Fluorescence Ubiquitin Cell Cycle Indicator (FUCCI) technology in combination with YAP1 downregulation. In these models, mice were subjected to unilateral ischemia reperfusion injury (IRI) or glycerol-induced rhabdomyolysis to induce AKI. Polyploid cells have been then characterized by single cell-RNA sequencing analysis, cell sorting, FACS analysis, super-resolution and transmission electron microscopy.
RESULTS: After AKI, YAP1 is activated triggering TC polyploidization. Polyploid TC increase in parallel to massive cell death triggered by AKI suggesting that polyploidization could be a means to escape cell death. Indeed, we found that polyploid TC tends to accumulate genome instability and survive, while diploid TC do not. Of note, virtually all dying cells were cycling cells based on the Fucci2aR reporter suggesting that TC death occurred during the S or G2/M phase. As polyploid TC increase immediately following AKI, they may be required to survive injury and damage by sustaining renal function. In order to evaluate the functional role of polyploid cells during AKI, we generated mice where YAP1 is knocked-out specifically in TC (YAP1ko mice). Indeed, after AKI, YAP1ko mice showed a reduced number of polyploid cells, worsened kidney function and a dramatic reduction of mouse survival, proving that polyploidization is required to survive AKI.
CONCLUSION: We demonstrated that (i) after AKI TC accumulate genome instability and die or become polyploidy; (ii) TC polyploidy is essential to preserve residual kidney function immediately after AKI.

MO289 IL33 EXERTS TOXICITY IN THE HEART AS SECRETED BY RENAL INFLAMMATION

Nans Florens[1], Rajesh Kasam[1], Valeria Rudman Melnick[2], Vikram Prasad[1], Scott N. Blair[1], Sui-Chin J. Lin[1], Allen York[1] and Jeffery D. Molkentin[1]

[1]Cincinnati Children's Hospital Medical Center, Heart Institute, Cincinnati, USA and [2]Division of Nephrology, Cincinnati Children's Hospital Medical Center, Cincinnati, USA

BACKGROUND AND AIMS: Acute kidney injury (AKI) is a major cardiovascular risk factor, regardless of the severity or the origin of the AKI. Among this risk, AKI imparts an array of signals and long-term physiologic alterations that can precipitate heart insufficiency and hypertrophy. However, few direct molecular mechanisms have been identified that show how AKI leads to cardiac disease and maladaptation. The aims of this project are to assess the role of IL33 in remote cardiac damage after AKI.
METHOD: AKI was induced in mice by ischemia–reperfusion injury of both right and left kidneys by 30min of reversible artery occlusion. To examine the role of renal inflammation more specifically, we used the unilateral ureteral obstruction (UUO) model, which does not cause acute injury. For UUO, an incision was made in the lower left quadrant of the abdomen and the left ureter was isolated and ligated permanently. Mice were treated to manipulate select signaling pathways with either Adenovirus-associated virus 9 (AAV9) or recombinant proteins. We generated AAV9 carrying the IL33 gene or an empty vector (EV) under a CMV promoter and injected 10^{12} viral particles intrathoracically in 6 to 9 days old pups. Recombinant IL33 (rIL33) or vehicle were injected intraperitoneally in IL33 KO adult mice after AKI (at D0, D1 and D2). Echocardiography was performed either at baseline or after 28 days of surgery. Hearts were fixed in 4% paraformaldehyde overnight and paraffin-embedded for fibrosis (picrosirius staining) and cardiomyocyte area measurement (WGA staining and ImageJ software). IL33 citrine reporter mice ($IL33^{cit}$) in the heterozygous state were used to show IL33 expression.
RESULTS: Our data showed that UUO and AKI were strong inducers of cardiac dysfunction after 28 days in adult mice, measured with echocardiography (mean ejectional fraction (EF): 58, 46, 47% in Sham, AKI and UUO group respectively, $P < .05$). Cardiac pathology was also present such as increased fibrosis in hearts from WT mice after AKI compared with Sham (2.3%, 4.5%, 8.3% of fibrosis area in Sham, AKI and UUO respectively, $P < 0.05$) and an increased mean cardiomyocyte area indicative of hypertrophy (333, 597, 1246 μm^2 in Sham, AKI and UUO respectively, $P < .05$). However, cardiac function and architecture were preserved either after 28 days of AKI or UUO in IL33 KO mice. In parallel, WT mice injected with AAV9-CMV-IL33 showed impaired cardiac function compared with mice injected with a control AAV9-empty vector after 8 weeks (EF 58% versus 42% in AAV-EV and AAV-IL33 respectively, $P < .05$). After AKI, AAV9-IL33 mice had even more impaired cardiac function compared to AAV-empty control mice (EF 42% versus 35% in AAV-EV and AAV-IL33 respectively). In IL33 KO mice, the injection of 1 µg of rIL33 in the early days of AKI restored cardiac dysfunction and fibrosis after 28 days of AKI (EF 59 versus 43% in Sham and AKI respectively, $P < .05$), yet vehicle injected mice were still protected. IL33 expression viewed with the Citrine reporter mice showed that it was locally downregulated in the heart after AKI, which was strictly localized in pericytes in the heart (PDGFRß+ cells). ELISA for protein levels of IL33 confirmed the reduction of this cytokine in hearts from WT AKI and UUO mice after 28 days compared with Sham (29.6, 4.2 and 2.7 fg of IL33/µg of protein in Sham, AKI and UUO hearts respectively, $P < .05$).
CONCLUSION: IL33 seems to exert toxicity in the heart as secreted by renal inflammation, which is against some previous reports in the literature where it was suggested to be a protective factor to the heart (whether released from the kidney or from within the heart).

MO290 INHIBITION OF THE KDM4C AND JMJD3 HISTONE DEMETHYLASES ALLEVIATES THE TUBULAR RENAL DAMAGE TRIGGERED BY ENDOPLASMIC RETICULUM STRESS

Paula Diaz-Ulnes[1,2], Maria Laura Saiz Alvarez[1,2], Viviana Corte[1,2], Aida Bernardo Florez[1], Cristian Ruiz Bernet[1], Carlos Lopez-Larrea[1,2,3] and Beatriz Suarez-Alvarez[1,2]

[1]Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Translational Immunology Laboratory, Oviedo, Spain, [2]RICORS2040 (Kidney Diseases), Institute of Health Carlos III,, Madrid, Spain and [3]Hospital Universitario Central de Asturias, Immunology Service, Oviedo, Spain

BACKGROUND AND AIMS: Alterations in protein homeostasis in tubular cells lead to endoplasmic reticulum (ER) stress activating the unfolded protein response (UPR) pathway, which contributes to repair or aggravate the renal damage [1]. This pathway is initiated by three major protein sensors (IRE1α, PERK and ATF6) that activate their corresponding transcription factors (TF), XBP1, ATF4 and ATF6, respectively, to ultimately regulate the transcription of numerous genes essential for cell survival. However, an exacerbated activation of the UPR pathway can also lead to the expression of genes related to inflammation and fibrosis, cell death or autophagy contributing to perpetuate the renal damage [2]. The balance between these two processes (adaptive/maladaptive response) is mediated not only by the