TWEAK/FN14 MEDIATES SECONDARY CELL DEATH DURING ACUTE KIDNEY INJURY

D. Martin-Sanchez[1,2], M. Fontecha-Barriuso[1,2], P-Cannata-Ortiz[5], MD. Sanchez-Niño[1,2], M-Ruiz-Ortega[1,2,3], J. Egido[1,3,4], A. Linkermann[6], A. Ortiz[1,2,3,4], AB. Sanz[1,2].

1.Nephrology Laboratory, IIS-Fundación Jiménez Díaz, Madrid, Spain. 2.REDinREN, Madrid, Spain. 3.School of Medicine, UAM, Madrid, Spain. 4.IRSIN, Madrid, Spain. 5.Pathology, IIS-Fundación Jiménez Díaz, School of Medicine, UAM, Madrid, Spain. 6.Division of Nephrology, Department of Internal Medicine III, University Hospital Carl Gustav Carus, Dresden, Germany.

diego.martin@fjd.es

INTRODUCTION:

Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK, TNFSF12) is a member of the TNF superfamily that induces renal tubular cell death in a proinflammatory environment. TWEAK, TNFα and IFNγ (TTI)-induced cell death has features of apoptosis and is associated to activation of caspases and mitochondria stress. However, inhibition of caspases with zVAD in vitro and in vivo was not protective. Recently, it was reported that ferroptosis plays a key role in the initial wave of tubular cell death in folic acid-induced acute kidney injury (AKI), and beyond cell death, it mediates upregulation of inflammatory proteins, including Fn14, the TWEAK receptor.

AIM:

We hypothesized that TWEAK/Fn14 axis could contribute to a secondary wave of inflammation-related cell death during AKI.

METHODS:

Animal model: All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory and were approved by the animal ethics committee of IIS-FJD. C57BL/6 mice wild type (WT) or Fn14 knock out (Fn14-KO) from Biogen Idc. (Cambridge, MA) (12- to 14-week-old, 5 per experimental group) received a single intraperitoneal (i.p) injection of folic acid 250mg/kg in 0,3mol/l sodium bicarbonate or vehicle, and were sacrificed 24 and 72 hours later.

Cells and reagents: MCT cells were cultured in RPMI 1640 (GIBCO, Grand Island, NY), 10% heat-inactivated fetal calf serum, 2 mM glutamine, 100 U/mL penicillin and 100µg/mL streptomycin, in 5% CO2 at 37°C. Cells were stimulated with 100ng/mL TWEAK (Millipore, CA), 30 ng/mL TNFα, and 30U/mL INFγ (Prepotech). zVAD-fmk (BD Bioscience, San Jose, CA, USA) and Necrostatin-1 (SIGMA, St. Louis, MO, USA) were dissolved in DMSO and added 1hour prior to stimuli.

Western Blot: Samples homogenized in lysis buffer were separated by 10-12% SDS-PAGE under reducing conditions. Primary antibodies were anti-phsopho-MLKL(1:500 Abcam), anti-cleaved caspase 3 (1:1000, Cell Signaling) and anti-cleaved caspase 8 (1:1000, Cell Signaling). Blots were then probed with anti-α-tubulin (1:2000, Sigma) or anti-GAPDH (1:5000, Millipore) and levels of expression were corrected for minor differences in loading.

Mitochondrial membrane potential (MMP): MMP were determined by tetramethylrhodamine methyl ester (TMRM) fluorescence (Molecular Probes, Lifetechnologies). Fluorescence intensity was measured by flow cytometry using BD FACS Diva Software (BD Bioscience).

siRNA transfection: Cells were seeded and transfected on the following day with 20nM of a scrambled siRNA or siRNA against RIP3, MLKL or RIP1 by using lipofectamine reagent (Invitrogen).

Assessment of cell death: Cell death were analyzed by annexin V/7-AAD staining and cells counted by flow cytometry using BD FACS Diva Software (BD Biosciences). Nuclei morphology following treatment was examined by DAPI staining. Cell viability was estimated using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich) colorimetric assay.

Statistics: Statistical analysis was performed using SPSS 11.0 statistical software. Results are expressed as mean ± SEM. Significance at the p<0.05 level was assessed by nonparametric Mann–Whitney U tests for two groups and ANOVAs for three or more groups.

CONCLUSIONS:

The TWEAK/Fn14 axis plays a key role in secondary, inflammation-related cell death in AKI. In vitro experiment showed that TWEAK/TNFα/IFNγ (TTI)-induced cell death has features of apoptosis but is dependent on the kinase activity of RIPK1. By contrast, caspase inhibition changed the mode of cell death to necroptosis. These results open a new therapeutic window to treatment of AKI once established.

RESULTS:

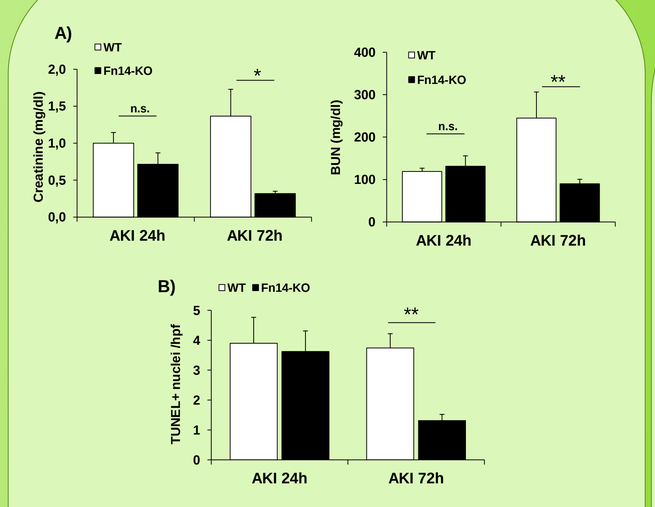

Figure 1. Fn14 absence reduces cell death in AKI. A) Renal function was assessed by plasma creatinine and BUN levels. Fn14-KO preserved renal function at 72h. *p<0,01, **p<0,004. **B)** Quantification of cell death. TUNEL positive nuclei, **p<0,004.

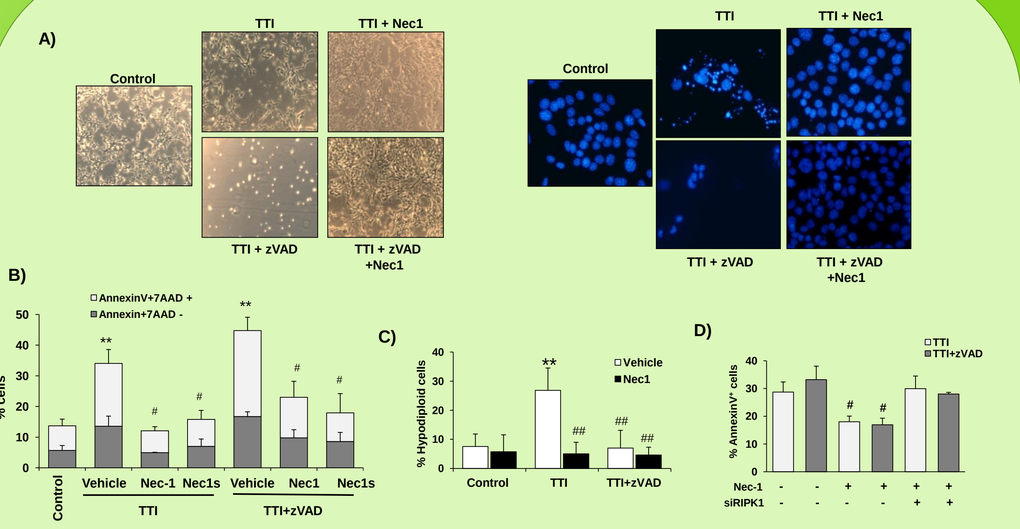

Figure 2. Effect of Nec-1 over cell death induced by TWEAK/TNFα/IFNγ (TTI) in vitro. Tubular cells exposed to TTI alone or in presence of zVAD (TTI/Z) were pre-treated with Nec-1or Nec-1s **A**) Contrast phase microscopy and DAPI photographs. **B)** Percentage of positive annexin V cells at 24h, **p<0,009 vs control, #p<0,02 vs vehicle. **C)** Hypodiploid cells were measured by flow cytometry of DNA content, **p<0,0001 vs control, ##p<0,004 vs TTI alone. **D)** Effect of absence of RIP1 over Nec-1 protection**.** Percentage of positive annexin V cells at 24h, #p<0,03 vs TTI or TTI/Z alone.

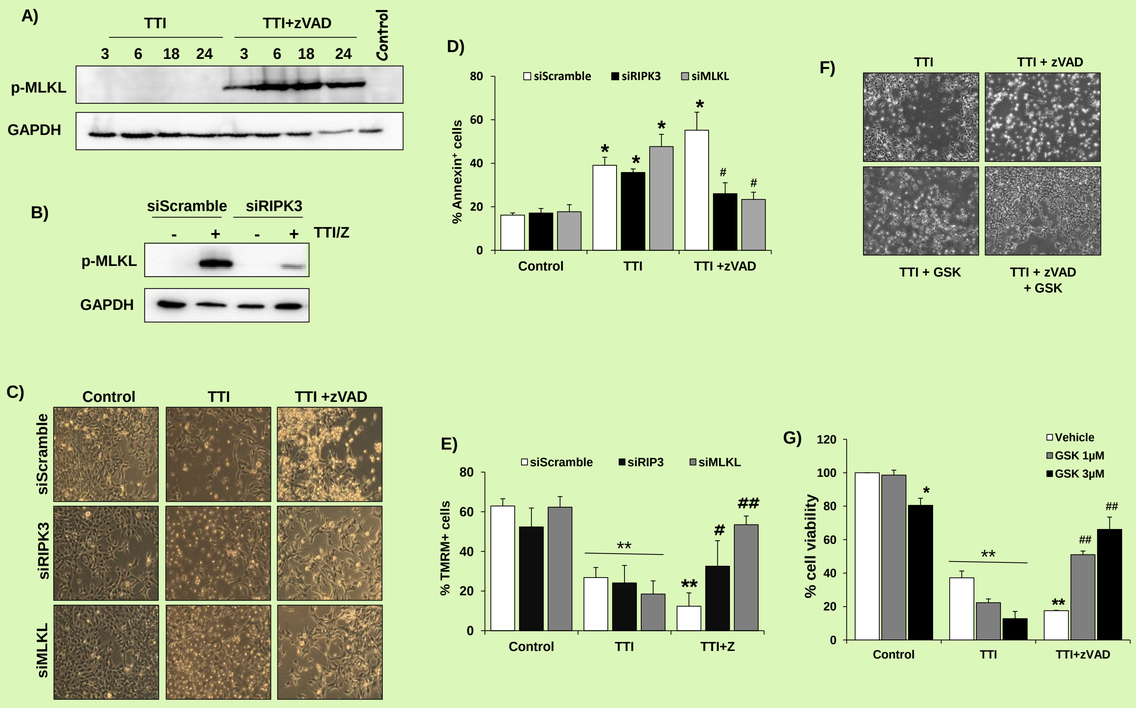

Figure 3. RIPK3 and MLKL play a key role in TTI/Z-induced cell. A) Time course of p-MLKL levels in tubular cells treated with TTI or TTI/Z. **B)** p-MLKL levels in tubular cells treated with TTI or TTI/Z in presence of siRNA Scramble or siRNA against RIPK3. **C, D, E)** RIPK3 or MLKL were targeted by an specific siRNA and cells were treated with TTI or TTI/Z, **(C)** contrast phase microscopy photographs, **D)** percentage of positive annexin V, *p<0.03 vs control, ##p<0.03 vs TTI/Z with siScramble. **E)** Loss mitochondrial membrane potential (MMP) was assessed by TMRM staining and flow cytometry **p<0,009 vs control, #p<0.04 vs, ##p<0.001 vs TTI/Z siScramble. **F, G)** Tubular cells were exposed to TTI/Z and pre-treated with an RIPK3 inhibitor, GSK, **(F)** contrast phase microscopy photographs, **(G)** Percentage of cell viability, **p<0,0001 vs control, ##p<0,0001 vs TTI.

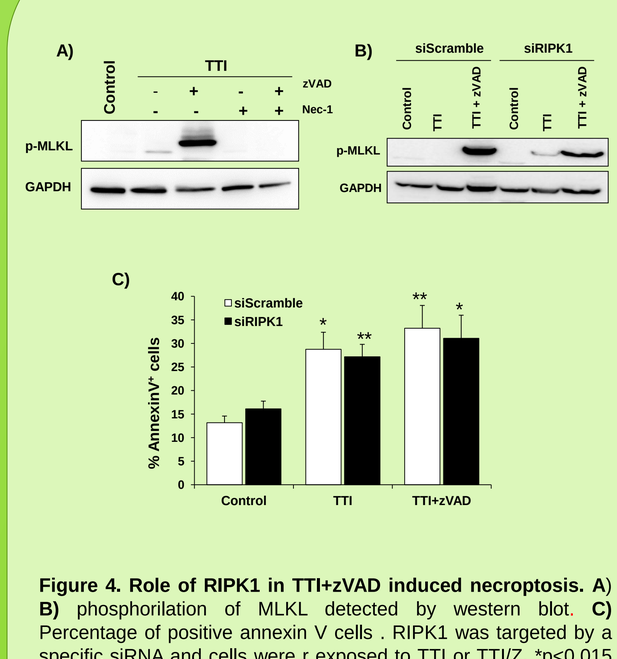

Figure 4. Role of RIPK1 in TTI+zVAD induced necroptosis. A) **B)** phosphorilation of MLKL detected by western blot. **C)** Percentage of positive annexin V cells . RIPK1 was targeted by a specific siRNA and cells were r exposed to TTI or TTI/Z, *p<0.015 vs control, **p<0.004 vs control.

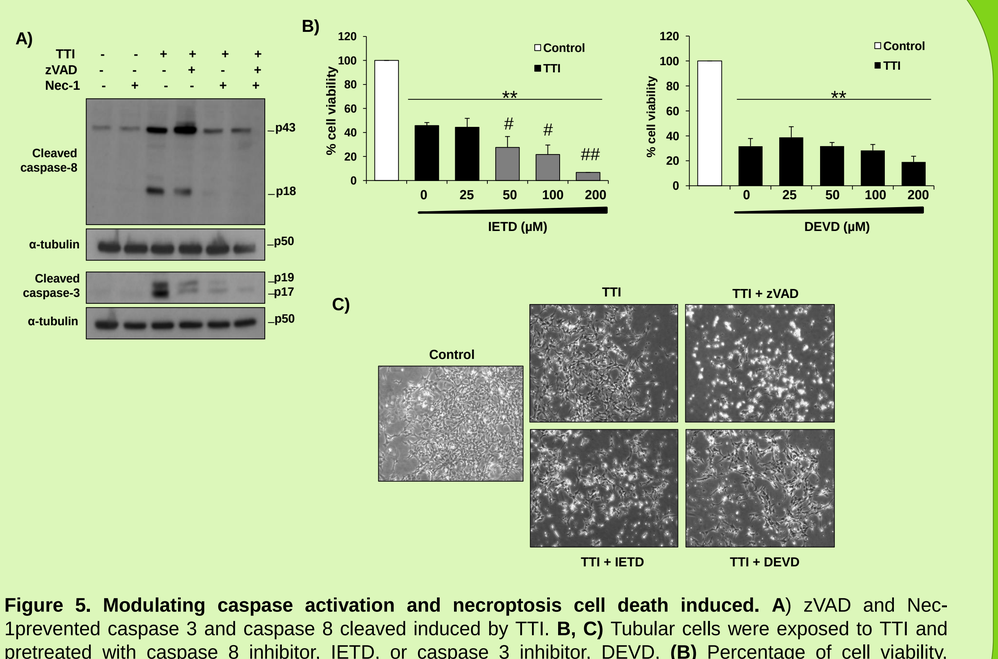

Figure 5. Modulating caspase activation and necroptosis cell death induced. A) zVAD and Nec-1prevented caspase 3 and caspase 8 cleaved induced by TTI. **B, C)** Tubular cells were exposed to TTI and pretreated with caspase 8 inhibitor, IETD, or caspase 3 inhibitor, DEVD, **(B)** Percentage of cell viability, **p<0.001 vs control, #p<0,05 vs 0 µM IETD, ##p<0,0001 vs vs 0 µM IETD,**(C)** contrast phase microscopy photographsat 24h.

54 ERA
244--MP
Experimental AKI II
Diego Martin-Sanchez
DOI: 10.3252/pso.eu.54ERA.2017
ePosters supported by F. Hoffmann- La Roche Ltd.
54th ERA-EDTA CONGRESS Madrid, Spain June 3rd-6th 2017
PosterSessionOnline.com