KSN 2016 Abstract Submission

Acute Kidney Injury

KSN2016ABS-1208

GADD45G PREVENTS ACUTE RENAL TUBULAR CELL NECROSIS.

Gyu-Tae Shin*, Hwa-Jung Lee, Heungsoo Kim

Background: GADD45G (growth arrest and DNA damage) is a molecule which responses to environmental stresses by checking on the cell cycle and the induction of apoptosis. In this study, we investigated for the first time the role of GADD45G in acute renal tubular cell injury.

Methods: Cultured primary human renal tubular epithelial cells (HRE) were infected with recombinant adenoviruses harboring GADD45G or LacZ for 48 h followed by cisplatin treatment. GADD45G KO mouse on a mixed 129/C57BL/6 background were backcrossed to C57BL/6 and phenotypic female mice were used. Acute kidney injury (AKI) was induced by intraperitoneal injection of cisplatin or lipopolysaccharide (LPS). Mouse renal tubular epithelial cells (MRE) were isolated from mouse kidneys. Cellular apoptosis and necrosis were detected using flow cytometry after annexin V-FITC and propidium iodide staining. Apoptotic cell death in tissue was detected by TUNEL staining.

Results: GADD45G overexpression itself was toxic to HRE cells by inducing apoptosis and necrosis. After cisplatin treatment (25 uM-200 uM), however, cellular necrosis was significantly reduced even though apoptosis was increased by GADD45G overexpression, resulting in improved survival of HRE cells. Consistently, MRE cells from GADD45G KO mice showed more necrosis and less apoptosis after cisplatin treatment (1.25uM-10 uM) compared with WT cells. To evaluate how such in vitro findings are applied in in vivo, AKI was induced in mice by administrating cisplatin or LPS. At 24 h and 48 h after cisplatin injection (20ug/g), serum creatinine was significantly higher in KO mice than WT mice. Urinary NGAL, a biomarker of kidney injury, was significantly higher in KO mice than WT mice. Similarly, serum creatinine was significantly higher and 24 creatinine clearance was significantly lower in KO mice than WT mice 24 h after LPS injection (10ug/g). PAS staining of kidneys showed significantly increased tubular necrosis manifest by tubular dilatation, vacuolated cells and tubular casts in KO mice compared with WT mice after cisplatin or LPS injection. In contrast, TUNEL positive apoptotic cells were rarely observed in both KO and WT mouse kidneys. Whereas cleaved caspases were significantly increased in cisplatin treated cells, these markers of apoptosis were not detected in kidney tissues. Collectively, GADD45G has the double-edged sword property, anti-necrotic and pro-apoptotic, which favorably acts in cisplatin- and LPS-induced AKI where necrosis is the main feature of kidney damage. In cisplatin

treated HRE cells, the up-regulation of pro-inflammatory cytokines including TNF, CX3CL1 and IL-8 were significantly prevented by GADD45G.

Conclusion: GADD45G prevents cisplatin-induced necrosis of renal tubular cells while promoting apoptosis. In AKI, GADD45G acts favorably in protecting kidney tubular cells.

Keywords: Apoptosis, GADD45g, necrosis, tubular cells