

Supplementary files

Renal tubular epithelial TRPA1 acts as an oxidative stress sensor to mediate ischemia-reperfusion-induced kidney injury through MAPKs/NF-κB signaling

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Table S1. Antibodies used for Western blot, and immunohistochemical staining; kits used for enzyme linked immunosorbent assay

| Target | Figure | Source | App |
|----------------|------------|---|---------|
| TRPA1 | 1, 2, 5, 6 | Rabbit Ab, Novus Biologicals (Littleton, CO, USA) | IHC, WB |
| 8-OHdG | 1 | Mouse Ab, Abcam (Cambridge, UK) | IHC |
| β -actin | 2, 5, 6 | Mouse Ab, Proteintech Group (Chicago, IL, USA) | WB |
| ERK | 9 | Rabbit Ab, Proteintech Group (Chicago, IL, USA) | WB |
| c-JNK | | | |
| P65 | | | |
| H1 | | | |
| Phospho-ERK | 9 | EnoGene Biotech (New York, NY, USA) | WB |
| Phospho-JNK | | | |
| Mouse NGAL | 4 | Bioporto (Hellerup, Denmark) | ELISA |
| Mouse MCP-1 | 4 | PeproTech (Rocky Hill, NJ, USA) | ELISA |
| Mouse MIP-2 | 4 | MyBioSource (San Deigo, CA, USA) | ELISA |
| Human IL-8 | 6,9 | Koma Biotech (Seoul, South Korea) | ELISA |

Abbreviations: TRPA1, transient receptor potential ankyrin 1; 8-OHdG, 8-hydroxy-2-deoxyguanosine; ERK, extracellular signal-regulated kinases; c-JNK, c-Jun N-terminal kinases; P65, a subunit of NF- κ B transcription complex; H1, histone protein 1; NGAL, neutrophil gelatinase-associated lipocalin; MCP-1, monocyte chemoattractant protein 1; MIP-2, macrophage inflammatory protein 2; IL-8, interleukin-8; Ab, antibody; App, application; WB, Western blot; IHC, immunohistochemical staining; ELISA, enzyme linked immunosorbent assay

Table S2. List of reagents, vehicles used to dilute the tested drugs, and companies

| Reagents | Vehicles | Companies |
|-------------------------------------|------------------|---|
| EGTA | NaOH | |
| NAC | H ₂ O | Sigma Aldrich (Merck KGaA, Darmstadt, Germany) |
| Apocynin | DMSO | |
| BAY11-7085 | | |
| HC-030031 | | Cayman Chemical (Ann Arbor, MI, USA) |
| PD98059 | | Calbiochem (Merck KGaA, Darmstadt, Germany) |
| SP600125 | | |
| DMEM | | Corning Mediatech, Manassas, VA, USA |
| Penicillin-streptomycin solution | | |
| FBS | | Thermo Fisher Scientific, Waltham, MA, USA |
| PAS staining kit | | Merck Millipore, Billerica, MA, USA |
| siRNA transfection reagent | | GE Healthcare Life Sciences |

Abbreviations: EGTA, ethylene glycol-tetraacetic acid; NAC, N-acetyl-cysteine; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum; PAS, periodic acid-Schiff; DMSO, dimethyl sulfoxide; N/A, not available.

Table S3. The clinical information of the biopsy-proven ATN patients.

| No | Age | Gender | DM | HTN | AKIN | TIS | TRPA1 expression | 8-OHdG expression |
|-------------|---------------|------------|------------|------------|-------------|-------------|------------------|-------------------|
| 1 | 73.8 | 1 | 0 | 0 | 3 | 3 | 0.244 | 0.253 |
| 2 | 70.3 | 1 | 1 | 1 | 3 | 4 | 0.356 | 0.267 |
| 3 | 35.5 | 1 | 0 | 0 | 3 | 4 | 0.341 | 0.263 |
| 4 | 35.2 | 1 | 0 | 0 | 3 | 4 | 0.312 | 0.255 |
| 5 | 19.4 | 0 | 0 | 0 | 2 | 4 | 0.312 | 0.252 |
| 6 | 69.6 | 1 | 1 | 0 | 3 | 2 | 0.215 | 0.229 |
| 7 | 73.4 | 0 | 0 | 1 | 1 | 2 | 0.194 | 0.242 |
| 8 | 72.3 | 1 | 0 | 1 | 3 | 1 | 0.194 | 0.218 |
| 9 | 58.9 | 0 | 0 | 0 | 1 | 1 | 0.182 | 0.235 |
| 10 | 56.0 | 1 | 1 | 0 | 2 | 1 | 0.152 | 0.204 |
| Data | 56.4 ±19.6 | 7 (70%) | 7 (70%) | 3 (30%) | 2.4 ±0.8 | 2.6 ±1.3 | 0.250 ±0.073 | 0.241 ±0.020 |

The codes of binary variables are that gender (male=1, female=0), DM (patients with history of type 2 DM=1, patients without type 2 DM=0), HTN (patients with history of HTN=1, patients without HTN=0), respectively. AKIN: Acute kidney injury network for classification of severity of acute kidney injury (AKI); TIS: tubular injury score; Data are expressed as *n* (%) for categorical data and as mean ± standard deviation for continuous data in the Data Row.

Table S4. List of the siTRPA1 component

| Name | Target Sequences (sense) | Antisense |
|-------------|--------------------------|---------------------|
| D-006109-01 | GAAGGACGCUCUCCACUUA | UAAGUGGAGAGCGUCCUUC |
| D-006109-02 | GGACAAUGGUGCACAAUA | UAUUUGUGCACCAUUGUCC |
| D-006109-03 | GGCAAUAAAUGUGCAAUA | UAAUUGCACAUUUAUUGCC |
| D-006109-04 | UCAAAGAGCUGGUAAUGGA | UCCAUUACCAGCUCUUUGA |