Poster SP053

CYSTEINE-RICH PROTEIN 61 MEDIATES KIDNEY FIBROSIS AFTER ISCHEMIA REPERFUSION INJURY

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Background

Clinical studies have demonstrated the risk of chronic kidney disease (CKD) after the occurrence of an acute kidney injury (AKI). Experimental works indicate that AKI result in incomplete repair, persistent tubulointerstitial inflammation and fibrosis. Cysteine-rich protein 61 (Cyr61), a secreted matrix-associated protein, has been found to be up-regulated in the kidney ischemia reperfusion injury (IRI) animal model. The present study aimed to investigate the role of Cyr61 in the kidney after IRI.

Method

1. Unilateral kidney IRI was induced by ligation of the left ureter in 8-week-old male ICR mice.
2. In vitro studies using normal rat kidney proximal tubular epithelial cells (NRK-52E).
3. Blockade of Cyr61 in unilateral IRI mice by treating polyclonal anti-Cyr61 antibody or non-specific IgG. Neutralizing anti-Cyr61 antibody.
4. Q-PCR, Western blots, Immunofluorescence stains, and picrosirius red stains.

Result

Figure 1. Cyr61 increased in the kidneys after unilateral IRI. (A) Q-PCR, (B) representative images and (C) bar chart of Western blot for Cyr61 following unilateral IRI, *P<0.05 vs. sham; #P<0.05 vs. contralateral (CLK) kidney. (D) IF staining showed Cyr61(+) cells are co-labelled with LTL, but not DBA. Cyr61 staining is also presented in KIM-1(+) cells (yellow arrows), as well as KIM-1(-) cells (white arrowheads).

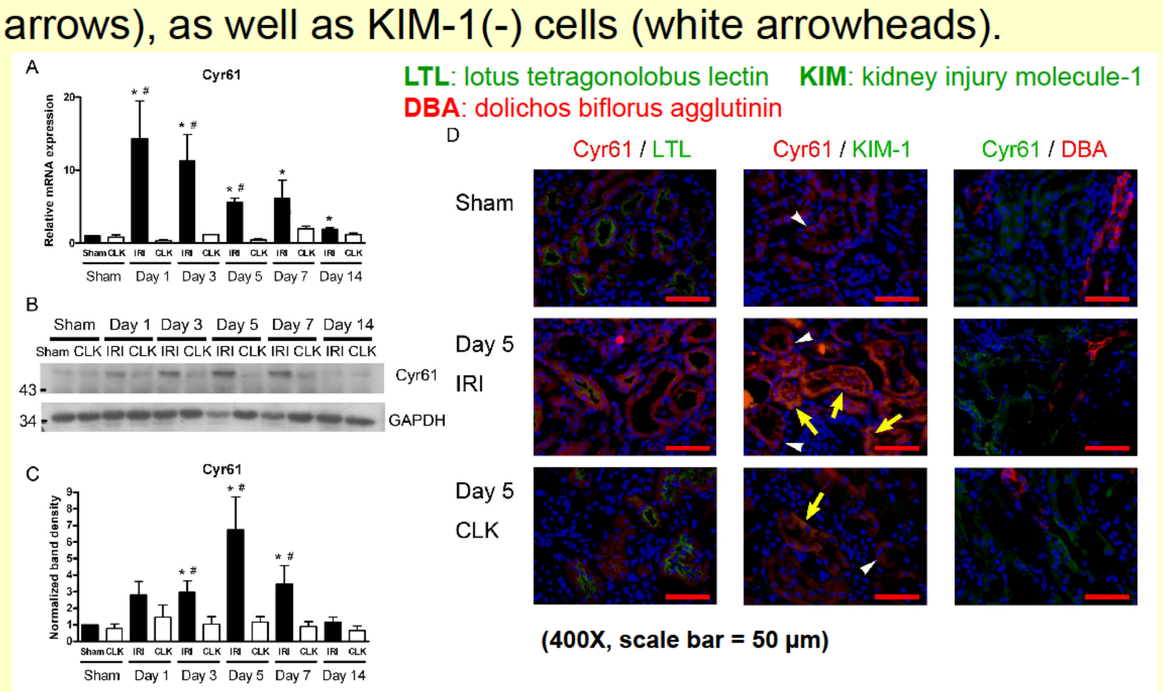


Figure 2. Cyr61 expression in cultured renal tubular epithelial cells under hypoxia. NRK-52E cells were incubated under normoxic or hypoxic conditions for 24 hours. *Cyr61* transcripts were significantly increased in serum- and glucose-free medium under hypoxia conditions. *P<0.05.

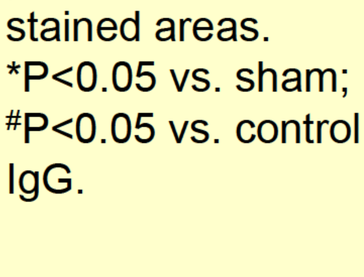


Figure 3. Effects of Cyr61 blockade in post-IRI kidney. Mice receive 10μg/gBW of control IgG (filled bar) or anti-Cyr61 antibody (open bar) at 2 hours before and everyday after IRI. (A) Urinary NGAL at 24 hours after IRI. (B)*IL-1β* and (C)*MCP-1* transcripts analyzed by Q-PCR. (D)IF staining for F4/80(+) macrophage in the kidneys. The bar chart summarizes the percentage of positive F4/80-stained areas. *P<0.05 vs. sham; #P<0.05 vs. control IgG.

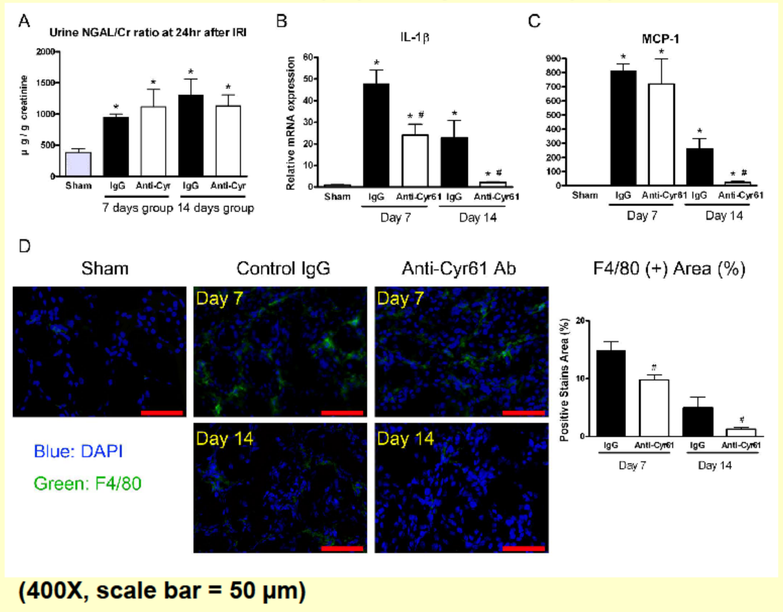


Figure 4. Cyr61 blockade attenuates fibrosis in the kidneys after unilateral IRI. Q-PCR shows (A) *Col 1-α1*, (B) *Col 3-α1*, and (C) *PAI-1* transcripts after IRI were inhibited by anti-Cyr61 antibody. (D) Representative images and (E) bar chart of Western blot showing blocking Cyr61 decrease α-SMA expression in the kidneys at 14 days. (F) Representative images and (G) bar chart summary of picrosirius red staining of post-IRI kidneys. *P<0.05 vs. sham operation; #P<0.05 vs. control IgG.

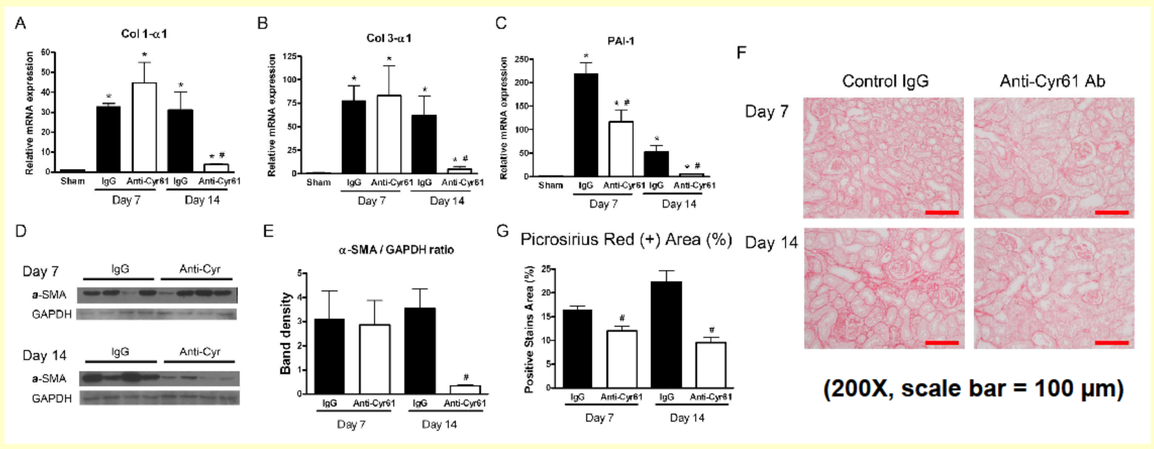
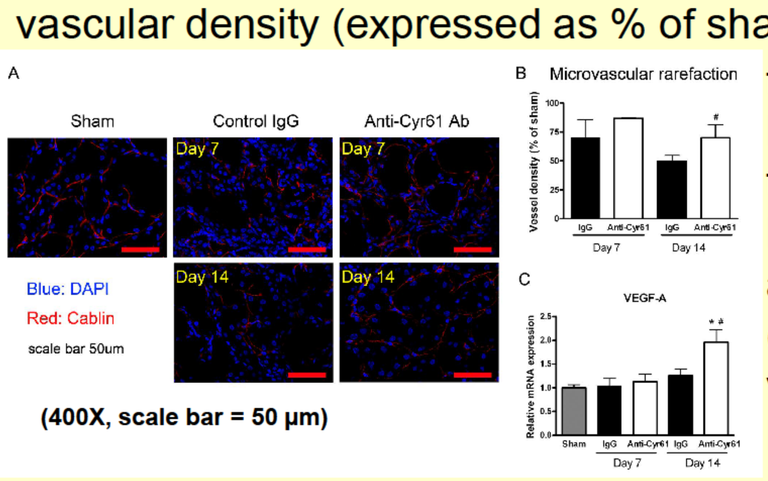


Figure 5. Cyr61 blockade prevent microvascular rarefaction in the kidneys after unilateral IRI. (A) IF staining for cablin shows kidney microvasculature (red) in post-IRI kidneys. (B) Morphometric quantification of kidney vascular density (expressed as % of sham) and (C) *VEGF-A* transcripts in post-IRI kidneys from mice treated with control IgG (filled bar) or anti-Cyr61 antibody (open bar).*P<0.05 vs. sham; #P<0.05 vs. IgG.



Conclusion

- **Cyr61 expression is increased in proximal tubular epithelial cells after ischemia-reperfusion kidney injury.**
- ***In vivo* Cyr61 blockade in post-IRI kidney reduces renal inflammation, ameliorates capillary rarefaction, and attenuates the severity of renal fibrosis.**
- **Cyr61 plays a role in the pathogenesis of AKI-CKD transition.**

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