Lung – Kidney Injury Cross-Talk

Mary E. Choi, M.D.
Associate Professor of Medicine
Joan and Sanford I. Weill Department of Medicine

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Introduction

• Significance of acute kidney injury (AKI) and chronic kidney disease (CKD) in critically ill patients

• Kidney-lung crosstalk: how kidney dysfunction can adversely affect distant organ function including the lungs

• Understanding the mechanisms involved in the pathogenesis of kidney injury is crucial in developing new therapeutic targets

• Transforming growth factor – beta 1 (TGF-β1): multifunctional cytokine
  - key mediator of kidney injury response

• Autophagy: process of “self-eating”
  - cytoprotective role in kidney injury
  - promising new therapeutic target to mitigate kidney injury and distal organ damage like lungs
**Defining Acute Kidney Injury (AKI)**

**RIFLE** *(Risk of renal dysfunction, Injury to the kidney, Failure or Loss of kidney function, and End-stage kidney disease)* **and** **AKIN** *(Acute Kidney Injury Network)* **criteria**

<table>
<thead>
<tr>
<th>RIFLE criteria</th>
<th>AKIN criteria</th>
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<tbody>
<tr>
<td><strong>Risk</strong></td>
<td></td>
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<tr>
<td>sCreatinine</td>
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<tr>
<td>↑ sCrea × 1.5</td>
<td>t sCrea × 1.5 or ↑ ≥ 0.3 mg/dl in sCrea</td>
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<tr>
<td>Urine output criteria</td>
<td>Urine output criteria</td>
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<tr>
<td>&lt; 0.5 ml/kg per h× 6 h</td>
<td>&lt; 0.5 ml/kg per h× 6 h</td>
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<tr>
<td><strong>Injury</strong></td>
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<tr>
<td>sCreatinine</td>
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<td>↑ sCrea × 2</td>
<td>t sCrea × 2</td>
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<tr>
<td>Urine output criteria</td>
<td>Urine output criteria</td>
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<tr>
<td>&lt; 0.5 ml/kg per h× 12 h</td>
<td>&lt; 0.5 ml/kg per h× 12 h</td>
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<td><strong>Failure</strong></td>
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<tr>
<td>sCreatinine</td>
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<tr>
<td>↑ sCrea × 3 or ≥ 0.5 mg/dl if baseline sCrea ↑ &gt; 4.0 mg/dl</td>
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<tr>
<td>or anuria × 12 h</td>
<td>or anuria × 12 h</td>
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<tr>
<td><strong>Loss</strong></td>
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<tr>
<td>Complete loss of renal function &gt; 4 weeks</td>
<td>Patients who receive RRT are considered to have met stage 3 criteria, irrespective of the stage they are in at the time of RRT</td>
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<tr>
<td><strong>End-stage</strong></td>
<td></td>
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<tr>
<td>End-stage renal disease</td>
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Singbartl K et al. *Kidney Int* 2012;81:819-825
Epidemiology of AKI

Distribution and hospital mortality for each RIFLE category

Fx3 indicates patients with AKI and a three-fold increase in creatinine, Fc indicates patients with AKI and a creatinine >400 [μmol/L] that has not increased three-fold.

Epidemiology of AKI in ICU patients:

Acute Kidney Injury - Epidemiologic Prospective Investigation (AKI-EPI) study

A multinational cross-sectional study performed in 97 centers to evaluate occurrence of AKI in patients during the first week of ICU admission

AKI defined by Kidney Disease: Improving Global Outcomes (KDIGO) criteria


<table>
<thead>
<tr>
<th>Stage</th>
<th>Serum creatinine (Scr)</th>
<th>Urine output</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.5 to 1.9 times baseline or ≥0.3 mg/dl (≥26.5 μmol/l) increase</td>
<td>&lt;0.5 ml/kg/hour for 6 to 12 hours</td>
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<tr>
<td>2</td>
<td>2.0 to 2.9 times baseline</td>
<td>&lt;0.5 ml/kg/hour for ≥12 hours</td>
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<tr>
<td>3</td>
<td>3.0 times baseline or increase in Scr to ≥4.0 mg/dl (≥353.6 μmol/l) or initiation of renal replacement therapy or in patients &lt;18 years, a decrease in eGFR to &lt;35 ml/minute per 1.73 m²</td>
<td>&lt;0.3 ml/kg/hour for ≥24 hours or anuria for ≥12 hours</td>
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Hoste EA et al. *Intensive Care Med* 2015;41:1411-1423
Epidemiology of AKI in ICU patients: AKI-EPI study results

- AKI occurred in more than half of ICU patients (1032 out of 1802 ICU patients)
- Increasing severity of AKI was associated with increased mortality

Hoste EA et al. Intensive Care Med 2015;41:1411-1423
Etiology of AKI

- Sepsis 271 (40.7 %)
- Hypovolemia 227 (34.1 %)
- Drug related 96 (14.4 %)
- Cardiogenic shock 88 (13.2 %)
- Hepatorenal syndrome 21 (3.2 %)
- Obstruction of the urine outflow tract 9 (1.4 %)
Mortality rate of sepsis syndrome patients: according to the pattern of change (either improvement, no change, or worsening) in severity of organ dysfunction

AKI is a frequent complication in critically ill patients, particularly those with sepsis, and is associated with increased mortality.

Despite advances in renal replacement therapy and supportive care, morbidity and mortality associated with AKI in the critically ill remain high.

Moreover, pre-existing chronic kidney disease (CKD) has marked impact on occurrence of AKI.

AKI can exacerbate progression of underlying CKD, or sometimes lead to new onset of CKD.

Kidney dysfunction can also adversely affect distant organ function including the lungs. It is now recognized that AKI-induced acute lung injury (ALI) extends beyond simple volume overload.
Lung – Kidney interactions during AKI

Alveolar cell apoptosis
Inflammation
↑ Vascular permeability

Mediators
Cytokines/chemokines
Oxidative stress
Uremic toxins
Activated neutrophils

↓ Renal perfusion
Blood gas disturbances
Inflammation/apoptosis

Mediators
Hypoxemia
Hypercapnia
PEEP
Biotrauma
CD11b+Ly6G+ neutrophils were increased not only in the kidneys, but also in the blood and the lungs following kidney injury induced by unilateral ureteral obstruction (UUO).
CD11b+Ly6G+ neutrophils were increased not only in the kidneys, but also in the blood and the lungs following kidney injury induced by unilateral ureteral obstruction (UUO)
• Thus, AKI can lead to multiple organ dysfunction through increase in circulating cytokines as well as activated leukocytes, resulting in the infiltration of cells into distant organ systems, including the lungs and cause ALI.

• Given that AKI can play a central role in the development of multiple organ failure, it is imperative for new therapeutic strategies that protect against AKI in order to improve the outcomes of critically ill patients.

• Understanding the mechanisms involved in the pathogenesis of kidney injury is crucial in developing new therapeutic targets.
Transforming Growth Factor-beta 1 (TGF-β1)

- Our investigations have centered on the pathophysiological role of a pleiotropic cytokine TGF-β1 as critical mediator of kidney injury.

- TGF-β1 regulates a wide variety of cellular functions:
  - Cell proliferation
  - Cell differentiation
  - Extracellular matrix synthesis

- While TGF-β1 is one of the most potent profibrotic cytokine, our work supports a provocative paradigm that TGF-β1 can exert paradoxical cytoprotective effects.
Final Common Pathway of Response to Injury

- **↑ Matrix proteins**
- **↓ Proteases**
- **↑ Protease Inhibitors**
- **↑ Integrins**
- **↑ TGF-β1**

**EXTRACELLULAR MATRIX ACCUMULATION**

- **↓**

**KIDNEY FIBROSIS**

- **↓**

**END STAGE KIDNEY**
TGF-β Signaling via receptors Type I (TβRI) and Type II (TβRII)
Autophagy is an evolutionarily conserved process that cells use to degrade and recycle cellular proteins and remove damaged organelles.

Choi AMK et al. *NEJM*, 2013
The molecular process of autophagy

(A) **Macroautophagy** (or autophagy) sequesters and degrades cellular organelles and protein aggregates through the sequential formation of autophagophores, autophagosomes and autolysosomes.

(B) **Microautophagy** degrades cellular organelles and protein aggregates by direct lysosomal engulfment.

(C) **Chaperone-mediated autophagy** (CMA) selectively degrades proteins containing KFERQ motif through the cooperation of heat shock cognate protein of 70 kDa (hsc70) and lysosome-associated membrane protein 2A (LAMP-2A).

Roles of Autophagy in Health and Disease

Mizushima N et al. Cell 2011
Molecular Regulation of Autophagy

- ATG5/Atg12 conjugation system
- LC3 conjugation system
- Isolation Membrane
- Vesicle Elongation
- Autophagosome
- Lysosome
- Autolysosome

Key Components:
- Atg6 / Beclin1
- LC3 conjugation system
- Atg5
- Atg12
- Atg16
- LC3-Gly
- Atg4
- Atg7
- Atg10
- Atg12
- Atg16
- UVRAG
- Beclin-1
- Vps34
- P
- Bcl2/Bcl-XL
- Vps15
- Bcl2/Bcl-XL
- LC3-I
- LC3-II

NewYork-Presbyterian
Weill Cornell Medical Center
**In vivo experimental model of kidney injury: unilateral ureteral obstruction (UUO)**

- A widely used model of kidney injury and progressive kidney fibrosis
- Induced by ligation of the left ureter
- Acute and complete ureteral obstruction leads to increased ECM deposition and tubulointerstitial fibrosis


Masson trichrome staining of kidney sections
Kidney cortex slices

Differential sieving technique

Collagenase digestion

Plating of cells
TGF-β1 stimulation induces autophagy in renal tubular epithelial cells

A

<table>
<thead>
<tr>
<th>TGF-β1</th>
<th>Baf</th>
<th>Beclin 1</th>
<th>LC3-I</th>
<th>LC3-II</th>
<th>β-actin</th>
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Human renal tubular epithelial cells (HK-2)

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<tr>
<th>TGF-β1</th>
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Primary mouse renal tubular epithelial cells (RTEC)

C

RTEC obtained from GFP-LC3 transgenic mice
TGF-β1 stimulation induces autophagy in renal tubular epithelial cells

Autophagy promotes intracellular degradation of type I collagen induced by TGF-β1


Autophagy is induced in renal epithelial cells following kidney injury induced by UUO.

**A**

- Beclin 1
- LC3-I
- LC3-II

**B**

- Sham  Con 3d  Con 7d  UUO 3d  UUO 7d  UUO 10d  UUO 14d
- GFP-LC3
- α-SMA
- Merge

Deletion of LC3 results in enhanced collagen deposition in the kidneys following UUO.
Heterozygous deletion of beclin 1 is associated with increased tubular epithelial cell apoptosis in kidneys following UUO

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<th>Sham</th>
<th>UUO</th>
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<tr>
<td>Beclin 1&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>Caspase-3</td>
<td>Caspase-3</td>
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<tr>
<td>Beclin 1&lt;sup&gt;+-/-&lt;/sup&gt;</td>
<td>β-actin</td>
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B. 

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<td>Beclin 1&lt;sup&gt;+/+&lt;/sup&gt;</td>
<td>Number of TUNEL positive cells</td>
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<td>Beclin 1&lt;sup&gt;+-/-&lt;/sup&gt;</td>
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Autophagy suppresses mature TGF-β levels in the kidneys following UUO

Expression of proTGF-β and mature TGF-β proteins in the kidney lysates from LC3+/+ and LC3−/− mice 7 days after UUO, or sham-operation. *p<0.01 versus sham mice; **p<0.05 versus LC3+/+ mice with UUO
Autophagy promotes degradation of mature TGF-β in renal tubular epithelial cells

A

B

C

D

E

 NIH3T3 fibroblasts
Schematic representation of proposed model: effects of autophagy induction in renal tubular epithelial cells on tubulointerstitial fibrosis

Activation of autophagy promotes degradation of mature TGF-β, and thereby decreasing TGF-β secretion, and suppresses tubulointerstitial fibrosis induced by UUO.
We uncovered that autophagy contributed to renal cell survival, by using both pharmacologic and genetic blockade of the autophagic pathway, via autophagic protein LC3 gene deletion ($LC3^{-/-}$) or LC3 siRNA.

TGF-β1 induced autophagy, via TAK1 and PI3K-Akt dependent pathway.

In mice deficient in autophagic protein Beclin 1 ($beclin\ 1^{+/−}$), we demonstrate that autophagy negatively regulates matrix production by promoting intracellular degradation of collagen and aggregated insoluble procollagen.

In UUO model of kidney injury, we demonstrated that autophagy regulates TGF-β1 expression and activation of autophagy promotes degradation of mature TGF-β, and thereby decreasing TGF-β secretion, and suppresses renal tubulointerstitial fibrosis.

Summary (3)
Our findings provide support for the cytoprotective role of autophagy in the kidney, and hold promise of a new therapeutic target against kidney injury and hopefully mitigate distal organ damage like lungs.
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