Over-Activation of Hypoxia-Inducible Transcription Factor 1 alpha (HIF)-1α by Chronic Hypoxia Mediates Chronic Ischemic Renal Injury

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Once the renal damage reaches a certain threshold, the progression of chronic renal disease is consistent and irreversible.

- Ultimately leads to fibrosis.
- Mechanisms are not yet known.

According to the United States Renal Data System:
- Total Medicare spending in 2006 - nearly $355 billion
- End Stage Renal Disease (ESRD) cost $23 billion.

Need efficient therapeutic strategies to reverse or prevent the progress of chronic renal injury.
Background

- **Hypoxia-inducible transcription factor 1 alpha (HIF-1α).**
  - Transcription factor.
  - Extremely prevalent in the kidney.
  - Hypoxia detected in all kinds of chronic renal diseases.
  - HIF-1α is up-regulated in different chronic renal diseases.
  - Activation of HIF-1α stimulates the fibrotic factors.
A

<table>
<thead>
<tr>
<th></th>
<th>Scrambled siRNA</th>
<th>ANG II + Scrambled siRNA</th>
<th>ANG II + HIF-1a siRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

![HIF1-α and β-actin bands](image)

B

<table>
<thead>
<tr>
<th></th>
<th>Naïve siRNA</th>
<th>Scrambled siRNA</th>
<th>Scrambled siRNA</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
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![Collagen I/II and TIMP-1 bands](image)

C

Collagen I/III level (normalized intensity)

![Bar chart](image)
Background

- Chronic hypoxia is possibly responsible for the over-activation of HIF-1α in chronic kidney diseases.
- HIF-1α may be a pathogenic factor that mediates chronic renal injury.
- At present, no direct evidence showing the contributing role of HIF-1α in this process.
- Therefore, in the present study we use 2 kidneys 1-clip rat as a chronic renal ischemia model to test our hypothesis, which is whether HIF-1α is increased in clipped kidneys and whether HIF-1α shRNA blocks renal injury.
Hypoxia

\[ \downarrow \]

HIF-1α

\[ \downarrow \]

Fibrogenic factors

\[ \uparrow \]

Extra Cellular Matrix

\[ \uparrow \]
Animal Model

Making a clip on the left renal artery

Plasmid transfection by intra renal artery injection
Bioluminescent Signal After Transfection of Luciferase Plasmids as Reporter genes.

Luciferin + O₂, ATP (Substrate) → Oxyluciferin + Light

Luciferase (Reporter Gene)
Blood Pressure Changes

Mean Artery Blood Pressure (mmHg) vs. Day after Surgery.

- **Ctrl**
- **LE**
- **HE**

ACEI administration is indicated at Day 10.
HIF-1α Expression in Each Group

A

HIF-1α

β-Actin

1 2 3 4 5 6 7 8

Ctrl L LE HE

B

HIF1a Level in Cortex

Ctrl=Normal Animal+ Luciferase,
L=Clipped Animal+Luciferase,
LE=Clip+ACEI+Luciferase,
HE=Clip+ACEI+HIF-1α shRNA
Glomerular Damage in Each Group

A

B

Glomerular Damage Index (GDI)

<table>
<thead>
<tr>
<th></th>
<th>Ctrl (N=4)</th>
<th>L(n=3)</th>
<th>LE(n=7)</th>
<th>HE(n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (±0.2)</td>
<td>3 (±0.3)</td>
<td>2 (±0.2)</td>
<td>1 (±0.2)</td>
</tr>
</tbody>
</table>
Effect of Silencing HIF-1α on Collagen Distribution

A

Collagen Staining Area Ratio (%)

Ctrl (n=4)   L (n=3)   LE (n=7)   HE (n=7)

B

LE

HE
Effect of Silencing HIF-1α on CD5, B Cell Marker

100X

Clip+ACEI+Luciferase  Clip+ACEI+HIF-1α shRNA  Clip+ACEI+Luciferase  Clip+ACEI+HIF-1α shRNA
Cortex  Medulla

200X
Conclusions

• Clip over-activates hypoxia-inducible transtription factor – 1 alpha (HIF-1α) expression by chronic hypoxia.

• Over-activation of HIF-1α contributes to chronic ischemic renal injury.

• Inflammation is involved in this renal injury.

• Silencing HIF-1α can protect chronic ischemic renal injury.
Future Directions

• Use disease models such as diabetic nephropathy, hypertensive nephropathy and see whether silencing HIF-1α can protect against chronic ischemic renal injury.

• HIF prolyl hydroxylase (PHD):
  • Oxygen sensor that regulate HIF-1α levels in response to changes of oxygen concentrations
  • In normoxia, targets HIF-1α for destruction.

• To determine whether PHD will also be involved in CKD via regulation of HIF-1alpha.
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