Role of MUC1 in Acute Kidney Injury and Recovery

REBECCA P HUGHEY
MUC1 is a glycoprotein expressed on the apical surface of polarized epithelial cells
- Localized to the distal tubule and collecting duct of kidney

Near perfect tandem repeats

(PD/ET/SRPAPGSTAPP/AAHGVTSA)

Autocatalytic cleavage within SEA module (sea urchin sperm protein, enterokinase and agrin)

Sequence highly conserved across species
- Multiple sites for kinase and adaptor docking, Cys-palmitoylation
MUC1 cyttoplasmic domain is a scaffold for protein docking

- Atypical nuclear localization motif
- *AP-2 clathrin adaptor binding
- *Grb2 binding to pYXNP/V
- *Cys dual palmitoylation
- p85 binds pYXXM
- PKCδ phosphorylates Thr41
- GSK-3β binding SXXXS site and phosphorylation of Ser44
- Tyr46 phosphorylation by EGFR, c-Src or Lyn kinases

CQCRRKNYGQLDIFPARDTYHPMSEYPTYHTHGRYVPPSSSTDSPYEKVSAGNGSSLSSYTNPAVAATSANL – COO-

\[ \beta - \text{catenin binding motif} \]
Growth of HK-2 cells under hypoxic conditions (1% O₂) produced a non-lethal but dysfunctional phenotype (8-36 h).

- Cell proliferation diminished (³H-thymidine uptake)
- No necrosis (LDH in medium)
- No apoptosis (annexin V staining)
- Morphology altered:
  - Decreased number of intercellular junctions (EM)
  - Increased paracellular permeability after 24 h (FITC-dextran)

- Microarray showed 48 genes induced by hypoxia including
  - HIF1α
  - VEGF
  - MUC1
- MUC1 gene has hypoxia-responsive elements for HIF1α binding
MUC1 is induced in a proximal tubule HK-2 cell line by hypoxia

<table>
<thead>
<tr>
<th>Time</th>
<th>8 h, n = 3</th>
<th>24 h, n = 3</th>
<th>96 h, n = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ctrl</strong></td>
<td><strong>Hypox</strong></td>
<td><strong>Ctrl</strong></td>
<td><strong>Hypox</strong></td>
</tr>
<tr>
<td>b-actin</td>
<td></td>
<td></td>
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<tr>
<td>MUC1 CT</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Fold diff.</td>
<td>3.5 ± 2.5</td>
<td>3.5 ± 1.0 *</td>
<td>3.8 ± 1.8 **</td>
</tr>
</tbody>
</table>
ACHN kidney cancer cell line

MUC1 transcript and protein levels increased after 6-24 h hypoxia

HIF-1α dependent (siRNA)
MUC1-dependent invasion and migration (siRNA)

Rat model of ischemia-reperfusion (1 h clamp and 2 h recovery)

MUC1 transcript and protein levels increased in in collecting ducts and distal tubules (RT-PCR and IHC).
MOUSE MODEL OF ISCHEMIA-REPERFUSION INJURY

Mouse surgery by Tim Sutton from Indiana Univ School of Medicine

![Graph showing blood creatinine levels during ischemia-reperfusion injury. The y-axis represents blood creatinine (mg/dL) with values ranging from 0 to 3.0. The x-axis represents clamp time (0 min, 19 min, 25 min), and the graph shows a significant increase in blood creatinine after 25 minutes of clamp time. A 24 h recovery period is indicated.]
MUC1 IS PROTECTIVE IN A MODEL OF ISCHEMIA-REPERFUSION INJURY

Surgery by Tim Sutton from Indiana Univ School of Medicine

![Graph showing blood creatinine levels](image)
MUC1 IS PROTECTIVE IN A MODEL OF ISCHEMIA-REPERFUSION INJURY

Surgery by Tim Sutton from Indiana Univ School of Medicine

Mechanism of protection?
MUC1 is induced in a proximal tubule HK-2 cell line by hydrogen peroxide

TUMOR CELL LINES:
MCF-7 – MUC1 induced 2-3 fold in 15 min with 0.4 mM peroxide
ZR-75-1 – MUC1 induced 2-3 in 1-2 h with 0.4 mM peroxide
HCT116 that lack MUC1 – SOD1, SOD2, catalase and GSH peroxidase induced 2-3 fold by transfection with MUC1; cellular ROS reduced

Yin – Kufe 2003 J Biol Chem 278 35458-64

Does MUC1 play a role in recovery?
Open

Deletion of the epidermal growth factor receptor in renal proximal tubule epithelial cells delays recovery from acute kidney injury

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MUC1 IS FOUND IN A CO-IP WITH EGFR AFTER ISCHEMIA-REPERFUSION INJURY

Co-IP EGFR and MUC1 in mouse kidney extracts

<table>
<thead>
<tr>
<th>IP: EGFR</th>
<th>4h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>NT</td>
<td></td>
<td></td>
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<tr>
<td>shm I-R</td>
<td></td>
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</tr>
<tr>
<td>shm I-R</td>
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</tr>
</tbody>
</table>

| IB: EGFR |     |     |

| IB: MUC1 |     |     |

Kidney homogenate:
| IB: MUC1 |     |     |
MUC1 STIMULATES WOUND HEALING IN MDCK CELLS

LSP is a polymorphism of MUC1 (frequency 0.34) that alters splicing, produces a Long Signal Peptide, and ultimately adds nine amino acids to the N-terminus:

\[
\text{A P K P A T V V T} - \text{MUC1}
\]
APKPA TVVT – MUC1

Near perfect tandem repeats

(PD/ET/SRPAPGSTAPP/AAHGVTSA)

Autocatalytic cleavage within SEA module (sea urchin sperm protein, enterokinase and agrin)

Sequence highly conserved across species
Multiple sites for kinase and adaptor docking, Cys-palmitoylation

O-linked glycans

N-linked glycans

N

C

membrane
SUMMARY

MUC1 is protective against I-R injury
MUC1 is induced by I-R injury
MUC1 enhances wound healing in MDCK cells

MUC1 is induced by hypoxia in HK-2 cells
MUC1 is induced by hydrogen peroxide in HK-2 cells

COULD MUC1 BE A BIOMARKER FOR AKI?
COULD MUC1 BE A BIOMARKER FOR AKI?

CT-2 anti-MUC1 cytoplasmic tail

N-linked glycan

O-linked glycan

CT-2 ANTI-MUC1 CYTOPLASMIC TAIL
COULD MUC1 BE A BIOMARKER FOR AKI?

B27.29 anti-MUC1 tandem repeats

O-linked glycans

N-linked glycans

C

membrane
# Relevant MUC1 Activities in Tumors

- $\text{H}_2\text{O}_2$ induces MUC1
- MUC1 induces SOD, catalase, GSH Prx
- MUC1 reduces cellular ROS
- MUC1 induces PHD3
- PHD3 modifies HIF-1$\alpha$
- HIF-1$\alpha$ associates with VHL for Ub
- MUC1 substrate of EGFR
- MUC1 stabilizes EGFR and enhances signaling ERK1/2 and Akt

## Injury Recovery

## Role of MUC1 in Normal Kidney Protection?

**HK2 cells:**
- Does $\text{H}_2\text{O}_2$ induce MUC1?
- Does MUC1 induce enzymes and PHD3?
- Does MUC1 reduce ROS?

**I-R mouse model (MUC1 KO vs control):**
- MUC1 is protective
- Where does MUC1 increase? PT?
- Do enzymes increase? PHD3? HIF-1$\alpha$?
- Does human MUC1 rescue the KO?

## Repair?

**HK2 cells:**
- MUC1 enhances wound healing
- Does MUC1 co-IP with EGFR?
- Is ERK1/2 and Akt phosphorylation dependent on MUC1 (siRNA)?

**I-R mouse model (MUC1 KO vs control):**
- Does MUC1 co-IP with EGFR?
- MUC1-dependent ERK1/2 and Akt phosphorylation?
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