What is MUC1?

- Transmembrane glycoprotein with an extracellular mucin-like domain that yields protection from pathogens (bacteria and virus)

- Expressed during development on the apical surface of most epithelia

- In adults it is found in the collecting duct and distal tubule of the kidney

- Reported in the human proximal tubule after acute renal damage

- Not just a membrane-anchored mucin:
  Cytoplasmic domain exhibits multiple sites for protein docking and phosphorylation involved in signal transduction
  (e.g., β-catenin, Src-family kinases, GSK3β, EGFR)
MUC1 modulates EGF receptor membrane trafficking in tumor cells

**Mechanism of interaction:**
- MUC1 is a substrate for the EGFR
- Co-IP of MUC1 and EGFR is Gal-3 dependent

**EGFR ligands**
- HB-EGF enhances degradation
- TGF-α enhances recycling
- EGF associated with both paths

**MUC1 regulates EGF receptor trafficking**
- Internalization and recycling
- Ubiquitination
- Ligand-induced degradation
- Ligand-mediated signaling

**Does EGFR and MUC1 interact in kidney epithelia?**

Pachampalli et al 2007 Oncogene
Ramasamy et al 2007 Mol Cell
Normal kidney epithelia:
MUC1 and EGFR are in the distal tubule
MUC1 is apical
EGFR is basolateral
EGF precursor for EGF is apical

Tumors of epithelial origin:
Polarity is lost
MUC1 and EGFR interact

Acute renal tubule damage:
Cells dedifferentiate and proliferate
MUC1 re-appears in proximal tubule
Levels of EGF-ligands vary
MUC1 and EGFR could interact

MODEL SYSTEMS
HK-2 CELLS (human proximal tubule) grown in hypoxic cultures
AKI by ischemia-reperfusion in mice (Muc1 KO)
Growth of HK-2 cells under hypoxic conditions (1% O$_2$) produced a non-lethal but dysfunctional phenotype.

- Cell proliferation was diminished ($^3$H-thymidine uptake)
- No necrosis (LDH in medium)
- No apoptosis (annexin V staining)
- Morphology was altered:
  - Decreased number of intercellular junctions (EM)
  - Increased paracellular permeability after 24 h (FITC-dextran)
- Microarray showed 48 genes induced by hypoxia (including HIF1$_\alpha$, VEGF and MUC1)
- MUC1 gene has hypoxia-responsive elements for HIF1$_\alpha$ binding
MUC1, a New Hypoxia Inducible Factor Target Gene, Is an Actor in Clear Renal Cell Carcinoma Tumor Progression

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MUC1 transcript and protein levels increased in ACHN kidney cancer cell line after 6-24 h hypoxia

HIF1α dependent (siRNA)
MUC1-dependent invasion and migration (siRNA)

MUC1 transcript and protein levels increased in rat model of ischemia-reperfusion (1 h clamp and 2 h recovery) in collecting ducts and distal tubules (RT-PCR and IHC).
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>
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<td><img src="image" alt="Ctrl 8h" /></td>
<td><img src="image" alt="Ctrl 24h" /></td>
<td><img src="image" alt="Ctrl 96h" /></td>
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<td><img src="image" alt="Hypox 24h" /></td>
<td><img src="image" alt="Hypox 96h" /></td>
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</tbody>
</table>

- **b-actin**:
  - 3.5 ± 2.5
  - 3.5 ± 1.0 *
  - 3.8 ± 1.8 **

- **MUC1 CT**:
  - 3.5 ± 2.5
  - 3.5 ± 1.0 *
  - 3.8 ± 1.8 **

* Asterisk indicates significance level.
HK-2 cells maintain polarity under hypoxic growth

HK-2 cells were cultured for three days on porous supports before growth in 1% oxygen for 48 h. Cells subjected to staining for TJ, or surface biotinylation (sulfo-NHS-SS-biotin).

Nuclei stained with phenylene diamine mounting medium.
Polarized expression of MUC1 and EGFR is altered by hypoxia and correlates with co-IP

HK-2 cells were cultured for three days on porous supports before growth in 1% oxygen for 48 h. Cells subjected to surface biotinylation (sulfo-NHS-SS-biotin) or immoprecipitation and blotting.
Co-IP of MUC1 and EGFR in mouse kidney tissue after ischemia-reperfusion injury

Transgenic mice expressing human MUC1 in place of mouse Muc1 (Sandra Gendler, Mayo Clinic Scottsdale)

Pedicles of mouse kidneys were clamped for 23 min (surgery by Tim Sutton, Indiana University School of Medicine)

Mouse were sacrificed after 0, 4 or 24 h

Blood recovered for assay of creatinine (control data from Tim Sutton); kidneys sent to Pittsburgh
MUC1 is induced in rat kidney in an ischemia-reperfusion model

Pedicle of one rat kidney was clamped for 30 min

Rat was sacrificed after 24 h

Control was non-clamped kidney (surgery by Tim Sutton, Indiana University School of Medicine)

Microscopy of kidney cortical slices (Imagine Core, Pittsburgh Center for Kidney Research)

Ar, arteries
- PCT
- DCT
CONCLUSIONS

• MUC1 is induced by hypoxic conditions in a cell culture model of polarized proximal tubules (HK-2) and associates with EGFR (co-IP).

• MUC1 is induced by ischemia-reperfusion injury in both a mouse and rat model AKI
  MUC1 is induced in both distal and proximal tubules (rat IF)
  MUC1 associates with EGFR in mouse kidney tissue (co-IP)

FUTURE STUDIES

HK-2 cells:
Does the co-IP of MUC1 and EGFR reflect altered membrane trafficking and signaling due to growth in hypoxic culture?
What is the mechanism for the interaction? (galectins or palmitoylation)

Animal model
Does MUC1 expression alter injury and recovery?
(TgMUC1 vs Muc1 KO vs Gal-3 KO mice)
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