Pannexins in Acute Kidney Injury

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Pannexin1 - an ATP release channel

- Pannexin 1, a transmembrane protein belongs to a family of proteins exhibiting a structural homology to gap junction-forming invertebrate innexins.
- Pannexin 1 is the most ubiquitously expressed of the three pannexins. “Pan” “innexins” → pannexins.
- Large, non-selective transmembrane channels that efficiently release ATP to the extracellular space upon activation.
- Activated by caspase cleavage of their pore associated C-terminal tail that controls channel permeability.
- Normal physiological and pathophysiological functions - regulation of cell differentiation and migration, tissue development and regeneration, inflammation, wound healing and cell death.
Pannexin 1 Structure

- Connexin and pannexin share a similar structure, but no sequence homology.
- Connexin and pannexin form hemichannels
- Four transmembrane domains, two extracellular loops, one cytoplasmic loop, and cytoplasmic N- and C-terminal domains.
- Connexin channels can assemble into a gap junction that mediates intercellular communication
- Pannexin’s extracellular loop has a high level of glycosylation in mammalian cells, which prevent the formation of gap junctions.
Panx 1, extracellular ATP and Inflammation

- eATP serves as a danger signal
- eATP activates purinergic signaling, inflammasome formation
- Endothelial Panx1 facilitates leukocyte emigration
- Activation of Panx1 in immune cells facilitates their activation and migration
- eATP - CD39 and CD73 from the immune cells convert eATP into ADP, AMP, and adenosine which binds with A2a and A2b adenosine receptors and executes immunosuppression
PANX1 inhibitor, carbenoxolone, protects against kidney IRI in WT mice

CBX – carbenoxolone, 50μM, 200μL saline/mouse, IP, 1h before IRI
Global PANX1 deletion protects against renal IRI

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Panx1

- **Panx1**

Panx1/HPRT mRNA (A.U.)

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<th>+/+</th>
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Creatinine

- **Creatinine**

Plasma creatinine (mg/dL)

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Ngal

- **Ngal**

Ngal/Gapdh mRNA (A.U.)

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ATN Score

- **ATN Score**

ATN (% of outer medulla area)

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Panx1+/+

Panx1−/−
Global deletion of PANX1 prevents IRI-induced increase in leukocyte adhesion molecule expression.

**Icam1**

**Sele (E-selectin)**

**Selp (P-selectin)**
Parenchymal PANX1 deficiency is critical in protection against IRI

2-way Anova **** → P<0.0001
Proximal Tubule PANX 1 Deficient Mice are Protected from IRI

Pepck Cre mouse courtesy of Volker Haase
Endothelial PANX 1 Deficient Mice are Protected from IRI
IRI in Panx1 overexpressing mice

22 minutes bilateral IRI

24 minutes bilateral IRI

Plasma Creatinine (mg/dL)

WT OX

Plasma Creatinine (mg/dL)

WT OX Sham IRI
**In vitro** hypoxia-reoxygenation model

- TKPTS – mouse proximal tubule cells line
- Mineral oil
- ATP release
- Regular culture
- Hypoxia
- Reoxygenation

PANX1-deficient PT Cells release less ATP and express less TNFα after H/R

Intracellular ATP

Extracellular ATP

TNFα

CellTiter-Glo® Luminescent
PT Cells overexpressing PANX 1 release more ATP and express more TNFα after H/R
Summary: Intracellular changes during Panx1 deficiency

Panx1 deficiency in TKPTS cells
- Preserves cell survival in H/R in presence of oligomycin to reduce ATP synthesis.
- Nrf2, PGCα, TFAM are reduced.
- Reduced autophagic flux
  - ↓ LC3-1 to LC3-II conversion
  - ↑ p62
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