Acute Kidney Injury and Multi-Organ Dysfunction

Thomas Mueller

Division of Nephrology and Immunology
University of Alberta

KRESCENT – November 2011 Workshop
Montreal, Dec 10, 2011
Topics

1. Acute kidney injury and mortality
2. The transcriptome of the implant biopsy
3. Oncostatin M and its receptor in organ injury
Mortality in AKI has not changed much over time
Mortality of AKI

- Uncomplicated ARF: 5 - 10%
- ICU with multi-organ failure: 30 - 45%
- ICU with sepsis: 70 - 80%
AKI-induced distant organ effects

Initial sequencing and analysis of the human genome

International Human Genome Sequencing Consortium

Principle of microarrays

RNA fragments with fluorescent tags from sample to be tested

RNA fragment hybridizes with DNA on GeneChip

Affymetrix® info material
Looking at the stars
Analysis of microarray transcript data

Living donor kidneys (n=76)

Deceased donor kidneys (n=67)

Kidney biopsy

1 hour

Clinical data

1-2 days

Renal scan (DTPA or MAG3)

Microarray analysis

Genome Canada project
The transcriptome differentiates between living and deceased donor kidneys

The majority of the highest transcripts in deceased donor biopsies are associated with the acute phase response

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene description</th>
<th>acute phase ( \downarrow )</th>
<th>linfold DD vs LD</th>
<th>p-value</th>
<th>adj. p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>202376_at</td>
<td>SERPINA3</td>
<td>25.1</td>
<td>1.00E-26</td>
<td>7.39E-23</td>
<td></td>
</tr>
<tr>
<td>216238_s_at</td>
<td>FGB</td>
<td>16.2</td>
<td>3.34E-13</td>
<td>1.12E-11</td>
<td></td>
</tr>
<tr>
<td>219612_s_at</td>
<td>FGG</td>
<td>16.9</td>
<td>1.39E-13</td>
<td>5.26E-12</td>
<td></td>
</tr>
<tr>
<td>204988_at</td>
<td>FGB</td>
<td>11.3</td>
<td>1.06E-13</td>
<td>4.26E-12</td>
<td></td>
</tr>
<tr>
<td>221872_at</td>
<td>RARRES1</td>
<td>9.3</td>
<td>2.05E-16</td>
<td>2.07E-14</td>
<td></td>
</tr>
<tr>
<td>202018_s_at</td>
<td>LTF</td>
<td>7.2</td>
<td>5.32E-12</td>
<td>1.28E-10</td>
<td></td>
</tr>
<tr>
<td>206392_s_at</td>
<td>RARRES1</td>
<td>6.8</td>
<td>1.60E-17</td>
<td>2.27E-15</td>
<td></td>
</tr>
<tr>
<td>205650_s_at</td>
<td>FGA</td>
<td>6.6</td>
<td>2.03E-13</td>
<td>7.29E-12</td>
<td></td>
</tr>
<tr>
<td>215223_s_at</td>
<td>SOD2</td>
<td>6.0</td>
<td>4.64E-22</td>
<td>4.88E-19</td>
<td></td>
</tr>
<tr>
<td>230318_at</td>
<td>SERPINA1</td>
<td>6.0</td>
<td>3.62E-18</td>
<td>9.86E-17</td>
<td></td>
</tr>
<tr>
<td>205649_s_at</td>
<td>FGA</td>
<td>5.8</td>
<td>1.25E-10</td>
<td>1.93E-09</td>
<td></td>
</tr>
<tr>
<td>209752_s_at</td>
<td>REG1A</td>
<td>5.8</td>
<td>4.33E-11</td>
<td>7.74E-10</td>
<td></td>
</tr>
<tr>
<td>218641_s_at</td>
<td>SOD2</td>
<td>5.6</td>
<td>2.57E-20</td>
<td>1.26E-17</td>
<td></td>
</tr>
<tr>
<td>210652_s_at</td>
<td>C1orf34</td>
<td>5.5</td>
<td>2.05E-25</td>
<td>7.54E-22</td>
<td></td>
</tr>
<tr>
<td>202237_at</td>
<td>NNMT</td>
<td>5.5</td>
<td>6.32E-18</td>
<td>9.90E-16</td>
<td></td>
</tr>
<tr>
<td>214441_at</td>
<td>LBP</td>
<td>6.1</td>
<td>9.74E-12</td>
<td>2.16E-10</td>
<td></td>
</tr>
<tr>
<td>209774_x_at</td>
<td>CXCL2</td>
<td>4.7</td>
<td>7.17E-14</td>
<td>3.07E-12</td>
<td></td>
</tr>
<tr>
<td>225967_at</td>
<td>STEAP4</td>
<td>4.3</td>
<td>3.40E-18</td>
<td>6.59E-16</td>
<td></td>
</tr>
<tr>
<td>202075_s_at</td>
<td>PLTP</td>
<td>4.3</td>
<td>4.06E-11</td>
<td>7.32E-10</td>
<td></td>
</tr>
<tr>
<td>241981_at</td>
<td>FAM20A</td>
<td>4.1</td>
<td>4.31E-20</td>
<td>1.76E-17</td>
<td></td>
</tr>
<tr>
<td>202238_s_at</td>
<td>NNMT</td>
<td>4.1</td>
<td>5.49E-18</td>
<td>8.97E-16</td>
<td></td>
</tr>
<tr>
<td>204070_at</td>
<td>RARRES3</td>
<td>4.0</td>
<td>1.02E-20</td>
<td>8.63E-18</td>
<td></td>
</tr>
<tr>
<td>228017_s_at</td>
<td>C20orf56</td>
<td>4.0</td>
<td>2.21E-18</td>
<td>4.00E-16</td>
<td></td>
</tr>
<tr>
<td>210168_at</td>
<td>C6</td>
<td>3.9</td>
<td>6.11E-14</td>
<td>2.76E-12</td>
<td></td>
</tr>
<tr>
<td>203021_at</td>
<td>SLPI</td>
<td>3.8</td>
<td>9.66E-11</td>
<td>1.54E-09</td>
<td></td>
</tr>
<tr>
<td>212768_s_at</td>
<td>OLFM4</td>
<td>3.8</td>
<td>3.80E-11</td>
<td>6.93E-10</td>
<td></td>
</tr>
<tr>
<td>208470_s_at</td>
<td>HPR</td>
<td>3.7</td>
<td>3.09E-10</td>
<td>4.15E-09</td>
<td></td>
</tr>
<tr>
<td>227869_at</td>
<td>SOCS3</td>
<td>3.7</td>
<td>1.30E-09</td>
<td>1.47E-09</td>
<td></td>
</tr>
<tr>
<td>207526_s_at</td>
<td>ILIRL1</td>
<td>3.6</td>
<td>2.73E-13</td>
<td>9.36E-12</td>
<td></td>
</tr>
<tr>
<td>217767_at</td>
<td>C3</td>
<td>3.6</td>
<td>1.65E-13</td>
<td>6.11E-12</td>
<td></td>
</tr>
<tr>
<td>205000_at</td>
<td>DDX3Y</td>
<td>3.6</td>
<td>0.002412598</td>
<td>0.005933926</td>
<td></td>
</tr>
<tr>
<td>205466_s_at</td>
<td>HS3ST1</td>
<td>3.6</td>
<td>6.15E-13</td>
<td>1.93E-11</td>
<td></td>
</tr>
<tr>
<td>203645_s_at</td>
<td>CD183</td>
<td>3.5</td>
<td>5.54E-15</td>
<td>3.61E-13</td>
<td></td>
</tr>
<tr>
<td>220231_at</td>
<td>GPRX2</td>
<td>3.5</td>
<td>1.07E-12</td>
<td>3.10E-11</td>
<td></td>
</tr>
<tr>
<td>215078_at</td>
<td>SOD2</td>
<td>3.5</td>
<td>2.51E-13</td>
<td>8.83E-12</td>
<td></td>
</tr>
<tr>
<td>215049_x_at</td>
<td>CD183</td>
<td>3.4</td>
<td>1.50E-14</td>
<td>8.26E-13</td>
<td></td>
</tr>
<tr>
<td>228018_at</td>
<td>C20orf56</td>
<td>3.4</td>
<td>1.81E-15</td>
<td>1.34E-13</td>
<td></td>
</tr>
<tr>
<td>206697_s_at</td>
<td>HP</td>
<td>3.4</td>
<td>1.34E-09</td>
<td>1.51E-08</td>
<td></td>
</tr>
<tr>
<td>202357_s_at</td>
<td>BF</td>
<td>3.4</td>
<td>5.60E-14</td>
<td>2.56E-12</td>
<td></td>
</tr>
<tr>
<td>244657_at</td>
<td>NAD1</td>
<td>3.3</td>
<td>2.02E-08</td>
<td>1.85E-07</td>
<td></td>
</tr>
<tr>
<td>228804_at</td>
<td>FAM20A</td>
<td>3.3</td>
<td>7.45E-19</td>
<td>1.96E-16</td>
<td></td>
</tr>
<tr>
<td>206391_at</td>
<td>RARRES1</td>
<td>3.3</td>
<td>1.74E-14</td>
<td>9.37E-13</td>
<td></td>
</tr>
<tr>
<td>201042_at</td>
<td>TGM2</td>
<td>3.3</td>
<td>3.90E-21</td>
<td>3.18E-18</td>
<td></td>
</tr>
<tr>
<td>206504_at</td>
<td>CYP24A1</td>
<td>3.2</td>
<td>3.01E-08</td>
<td>2.33E-07</td>
<td></td>
</tr>
<tr>
<td>209869_s_at</td>
<td>STAT1</td>
<td>3.1</td>
<td>3.69E-12</td>
<td>9.23E-11</td>
<td></td>
</tr>
<tr>
<td>221477_s_at</td>
<td>SOD2</td>
<td>3.1</td>
<td>4.31E-23</td>
<td>6.02E-20</td>
<td></td>
</tr>
<tr>
<td>205729_at</td>
<td>OSMR</td>
<td>3.1</td>
<td>1.27E-14</td>
<td>7.30E-13</td>
<td></td>
</tr>
<tr>
<td>243206_at</td>
<td>RBCE1</td>
<td>3.1</td>
<td>7.31E-10</td>
<td>8.93E-09</td>
<td></td>
</tr>
<tr>
<td>222621_at</td>
<td>FGG</td>
<td>3.0</td>
<td>1.54E-18</td>
<td>3.43E-16</td>
<td></td>
</tr>
<tr>
<td>209588_at</td>
<td>PNMA2</td>
<td>3.0</td>
<td>7.23E-10</td>
<td>8.85E-09</td>
<td></td>
</tr>
</tbody>
</table>

Mueller T et al, AJT 2008
Topics

1. Acute kidney injury and mortality

2. The transcriptome of the implant biopsy

3. Oncostatin M and its receptor in organ injury
OSMR expression is associated with kidney injury biomarkers in 0-hr transplant biopsies grouped according to kidney function as measured by renal scan.
OSMR expression is increased with organ inflammation and injury

(Studies published in the GEO registry)

The red bar reflects the measured level of abundance of an individual transcript across the samples. The blue square represents rank order and gives an indication of where the expression of that gene falls with respect to all other genes on that array.

Kidneys of 1 week old mice who will develop polycystic kidneys due to lacking aquaporin-11 (AQP11)
Acute kidney injury model

- control
- sham
- experiment

- clamping of one kidney for 45 mins
- reperfusion for 24 hrs
- harvesting of
  - clamped kidney
  - non-clamped, contra-lateral kidney
  - heart
  - liver
  - lung
  - (brain)
  - spleen
  - blood
Ischemia-reperfusion injury induces Osmr and Ngal in local and distant organs in wild-type mice (n ≥ 3 mice per group)

* p < 0.05 control vs. clamped (clamp); +p<0.05 sham vs. clamp;
| p < 0.05 control vs. contra-lateral non-clamped kidney (contralat.);
| p < 0.05 clamp vs. contralat.
Impact of ischemic acute kidney injury
**BUN levels are significantly higher in WT compared to Osmr-/− mice**

**Plasma IL6 levels are higher in WT mice after unilateral renal ischemia**

*B*< 0.001 WT vs. Osmr-/−
Differences in Ngal and Il6 expression in local and distant organs in wild-type and Osmr-/- mice 24 hours after unilateral renal ischemia and reperfusion.

* p<0.03 WT sham vs other; † p < 0.001 Osmr-/- sham vs. other; # p < 0.02 WT clamp vs Osmr-/- clamp; ° p < 0.01 WT vs. Osmr-/- contralateral non-clamped kidney
Genes differentially expressed between sham operated and clamped kidneys in Osmr-/- mice and WT mice.
Pathways most differentially activated comparing WT vs. Osmr-/- mice
AKI is associated with
• systemic inflammation
• multi-organ dysfunction and
• high morbidity and mortality

Osmr-deficiency attenuates local and systemic inflammation

Sepsis is characterized by
• systemic inflammation
• multi-organ dysfunction and
• extremely high morbidity and mortality

? effect of Osmr-deficiency in sepsis?
Cecal ligation and puncture CLP

- 3-4 mo old C57/BL6 mice
- control
- sham
- experiment

- ligation of 50% of cecum
- puncture with either 18 or 25 G needle
- euthanasia at 18 and 24 hrs
- harvesting of
  - kidney
  - heart
  - liver
  - lung
  - spleen
  - blood
CLP induces Osmr and Ngal expression in WT mice in proportion to size of cecal puncture (18 gauge > 25 gauge) 
(n = 2 - 5 mice per group)

* p < 0.02 for control vs. combined CLP; + p < 0.02 control vs. 25G; o p < 0.02 control vs. 18G; ♦ p < 0.02 25G vs. 18G; ND – not determined, n = 2 in 18G
Mortality was significantly higher in WT mice (42%) compared to Osmr-/- mice (0%) 24 hours post CLP.
Differences in kidney Ngal, Il6 and BUN levels 24 hours after cecal ligation and puncture (18 G) in WT and Osmr−/− mice

**Ngal**

\[\text{%Hprt} \text{ for WT 18G and Osmr}^{-/-} \text{ 18G} \]

\[*p = 0.05 \text{ WT vs. Osmr}^{-/-}\]

**Il6**

\[\text{%Hprt} \text{ for WT 18G and Osmr}^{-/-} \text{ 18G} \]

\[P = \text{non-significant because of large variability in Il6 expression. Range 7.3 – 129 in WT and 0.02 -0.08 in Osmr}^{-/-}\]

**BUN**

\[\text{BUN mg/dL} \text{ for WT 18G and Osmr}^{-/-} \text{ 18G} \]

\[*p < 0.003 \text{ for WT vs. Osmr}^{-/-}\]
Acute Kidney Injury and Sepsis both induce a comparable inflammation and injury response as shown by Ngal across several organs.

Baseline expression in % Hprt

<table>
<thead>
<tr>
<th></th>
<th>KIDNEY</th>
<th>LUNG</th>
<th>HEART</th>
<th>KIDNEY</th>
<th>LUNG</th>
<th>HEART</th>
</tr>
</thead>
<tbody>
<tr>
<td>[6]</td>
<td>[702]</td>
<td>[10]</td>
<td>[8]</td>
<td>[697]</td>
<td>[33]</td>
<td></td>
</tr>
</tbody>
</table>
Thank You!

C Compston
BJ Pedrycz and D Grynoch
L Cairo and M Obeidat
WL Phan and Z Jacaj
LF Zhu and N Nation
R Khadaroo and her laboratory
V Mas and her group
VA Luyckx

University Hospital Foundation
Division of Nephrology
Topics

1. Acute kidney injury and mortality

2. The transcriptome of the implant biopsy

3. Oncostatin M and its receptor in organ injury
AKI-induced distant organ effects

Local injury induces distant organ injury

New biomarkers – renal troponins?
Acute tubular necrosis (ATN)

- Sloughed off debris in tubule lumen
- Healthy tubule
RIFLE classification

- **Risk**:
  - Increased creatinine $\times 1.5$ or GFR decrease $>25%$
  - Urine output criteria: $UO < 0.5 \text{ ml kg}^{-1} \text{ h}^{-1} \times 6 \text{ h}$

- **Injury**:
  - Increased creatinine $\times 2$ or GFR decrease $>50%$
  - Urine output criteria: $UO < 0.5 \text{ ml kg}^{-1} \text{ h}^{-1} \times 12 \text{ h}$

- **Failure**:
  - Increased creatinine $\times 3$ or GFR decrease $>75%$ or creatinine $\geq 4 \text{ mg per 100 ml (acute rise of } \geq 0.5 \text{ mg per 100 ml dl)}$
  - Urine output criteria: $UO < 0.3 \text{ ml kg}^{-1} \text{ h}^{-1} \times 24 \text{ h or anuria } \times 12 \text{ h}$

- **Loss**
  - Persistent ARF = complete loss of renal function $>4$ weeks

- **ESRD**
  - End-stage renal disease

Ricci et al, KI 2008
Tubular Epithelial Cell Injury and Repair

Ischemia/Reperfusion

Normal Epithelium → Loss of polarity → Cell death

Differentiation & Reestablishment of polarity

Proliferation

Sloughing of viable and dead cells with luminal obstruction

Migration, Dedifferentiation of Viable Cells

Adhesion molecules

Na⁺/K⁺-ATPase
How pattern recognition receptors induce innate immunity

Anders, Muruve. JASN 2011
Induction of a systemic inflammatory response

Tissue injury
pathogens +/- non-pathogens

release of
DAMPs

binding to
Receptors

activation of
Pro-inflammatory pathways

induction of
Cytokines / Chemokines / Type I interferons

Complement system

Coagulation system
Weekend Hospital Admission Associated With Increased Mortality Risk In AKI Patients.

In a press release, the American Society of Nephrology (4/15, pdf) wrote, "Patients with acute kidney injury (AKI) who are admitted to the hospital on a weekend are more likely to die than those admitted on a weekday, according to a study (pdf) appearing in an upcoming issue of the Journal of the American Society of Nephrology." After identifying "963,730 admissions with a diagnosis of AKI between 2003 and 2006," researchers found that "patients admitted with a primary diagnosis of AKI on a weekend had a 22% increased risk of dying by day three of admission, and a 7% increase of dying during the duration of their hospital stay, compared to patients on a weekday."

"The proportions of patients discharged to another short-term hospital (3.6% versus 3.4%, P<0.001) and to an intermediate care or skilled nursing facility (29.4% versus 28.5%, P<0.001) were greater for weekend than for weekday admissions," MedPage Today (4/15, Frieden) reported. In addition, "patients admitted on a weekend were...less likely to receive dialysis (OR 0.94, 95% CI 0.90 to 0.97), regardless of hospital size." MedPage added, "In an accompanying editorial (pdf), William McClellan, MD, of Emory University, in Atlanta, said the study 'is instructive and provides unexpected insight into hospital admissions for [acute kidney injury].'"

Medscape (4/15, MacReady) reported, "All in all, the authors concluded, 'these findings highlight the need to further investigate the availability and timing of provision of diagnostic and therapeutic strategies to patients hospitalized with AKI.'"
Topics

1. Acute kidney injury and mortality

2. The transcriptome of the implant biopsy

3. Oncostatin M and its receptor in organ injury
HE stained implant biopsies of living and deceased donors
The kidneys are clustered in relation to risk of delayed graft function (DGF)
Transcript levels of 10 acute phase response genes in implant biopsies
Distribution of all individual kidneys based on their transcriptome changes

“bad”

“good”
‘Outliers’ indicated by the transcriptome changes

DGF +++

BP ↓↓

sepsis

“bad”

“good”
The continuity of changes in kidney biopsies
The majority of the highest transcripts in deceased donor biopsies are associated with the acute phase response.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>acute phase</th>
<th>limfold DD vs LD</th>
<th>p-value</th>
<th>adj. p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>202376_at</td>
<td>SERPINA3</td>
<td>25.1</td>
<td>1.00E-26</td>
<td>7.39E-23</td>
<td></td>
</tr>
<tr>
<td>216238_s_at</td>
<td>FGB</td>
<td>16.2</td>
<td>3.34E-13</td>
<td>1.12E-11</td>
<td></td>
</tr>
<tr>
<td>219612_s_at</td>
<td>FGG</td>
<td>16.9</td>
<td>1.39E-13</td>
<td>5.26E-12</td>
<td></td>
</tr>
<tr>
<td>204996_at</td>
<td>FGB</td>
<td>11.3</td>
<td>1.06E-13</td>
<td>4.26E-12</td>
<td></td>
</tr>
<tr>
<td>221872_at</td>
<td>RARRES1</td>
<td>9.3</td>
<td>2.05E-16</td>
<td>2.07E-14</td>
<td></td>
</tr>
<tr>
<td>202018_s_at</td>
<td>LTF</td>
<td>7.2</td>
<td>5.32E-12</td>
<td>1.28E-10</td>
<td></td>
</tr>
<tr>
<td>206392_s_at</td>
<td>RARRES1</td>
<td>6.8</td>
<td>1.60E-17</td>
<td>2.27E-15</td>
<td></td>
</tr>
<tr>
<td>205650_s_at</td>
<td>FGA</td>
<td>6.6</td>
<td>2.03E-13</td>
<td>7.29E-12</td>
<td></td>
</tr>
<tr>
<td>215223_s_at</td>
<td>SOD2</td>
<td>6.0</td>
<td>4.64E-22</td>
<td>4.88E-19</td>
<td></td>
</tr>
<tr>
<td>230318_at</td>
<td>SERPINA1</td>
<td>6.0</td>
<td>3.62E-18</td>
<td>9.86E-17</td>
<td></td>
</tr>
<tr>
<td>205649_s_at</td>
<td>FGA</td>
<td>5.8</td>
<td>1.26E-10</td>
<td>1.93E-09</td>
<td></td>
</tr>
<tr>
<td>209752_at</td>
<td>REG1A</td>
<td>5.8</td>
<td>4.33E-11</td>
<td>7.74E-10</td>
<td></td>
</tr>
<tr>
<td>216041_s_at</td>
<td>SOD2</td>
<td>5.6</td>
<td>2.57E-20</td>
<td>1.26E-17</td>
<td></td>
</tr>
<tr>
<td>210652_s_at</td>
<td>C1orf34</td>
<td>5.5</td>
<td>2.05E-25</td>
<td>7.54E-22</td>
<td></td>
</tr>
<tr>
<td>202237_at</td>
<td>NNMT</td>
<td>5.5</td>
<td>6.32E-18</td>
<td>9.90E-16</td>
<td></td>
</tr>
<tr>
<td>214461_at</td>
<td>LBP</td>
<td>6.1</td>
<td>9.74E-12</td>
<td>2.10E-10</td>
<td></td>
</tr>
<tr>
<td>209774_x_at</td>
<td>CXCL2</td>
<td>4.7</td>
<td>7.17E-14</td>
<td>3.07E-12</td>
<td></td>
</tr>
<tr>
<td>225967_at</td>
<td>STEAP4</td>
<td>4.3</td>
<td>3.40E-18</td>
<td>6.59E-16</td>
<td></td>
</tr>
<tr>
<td>202075_s_at</td>
<td>PLTP</td>
<td>4.3</td>
<td>4.06E-11</td>
<td>7.32E-10</td>
<td></td>
</tr>
<tr>
<td>241981_at</td>
<td>FAM20A</td>
<td>4.1</td>
<td>4.31E-20</td>
<td>1.76E-17</td>
<td></td>
</tr>
<tr>
<td>202238_s_at</td>
<td>NNMT</td>
<td>4.1</td>
<td>5.48E-18</td>
<td>8.97E-16</td>
<td></td>
</tr>
<tr>
<td>204070_at</td>
<td>RARRES3</td>
<td>4.0</td>
<td>1.02E-20</td>
<td>6.83E-18</td>
<td></td>
</tr>
<tr>
<td>228017_s_at</td>
<td>C20orf58</td>
<td>4.0</td>
<td>2.21E-18</td>
<td>4.40E-16</td>
<td></td>
</tr>
<tr>
<td>210168_at</td>
<td>C6</td>
<td>3.9</td>
<td>6.11E-14</td>
<td>2.76E-12</td>
<td></td>
</tr>
<tr>
<td>203021_at</td>
<td>SLPI</td>
<td>3.8</td>
<td>9.68E-11</td>
<td>1.50E-09</td>
<td></td>
</tr>
<tr>
<td>213768_s_at</td>
<td>OLFM4</td>
<td>3.8</td>
<td>3.80E-11</td>
<td>6.93E-10</td>
<td></td>
</tr>
<tr>
<td>208470_s_at</td>
<td>HPR</td>
<td>3.7</td>
<td>3.00E-10</td>
<td>4.15E-09</td>
<td></td>
</tr>
<tr>
<td>227697_at</td>
<td>SOCS3</td>
<td>3.7</td>
<td>1.30E-09</td>
<td>1.47E-09</td>
<td></td>
</tr>
<tr>
<td>206526_s_at</td>
<td>IL1RL1</td>
<td>3.6</td>
<td>2.73E-13</td>
<td>9.36E-12</td>
<td></td>
</tr>
<tr>
<td>217767_at</td>
<td>C3</td>
<td>3.6</td>
<td>1.65E-13</td>
<td>6.11E-12</td>
<td></td>
</tr>
<tr>
<td>205000_s_at</td>
<td>DDX3Y</td>
<td>3.6</td>
<td>0.002412598</td>
<td>0.005933926</td>
<td></td>
</tr>
<tr>
<td>205466_s_at</td>
<td>HS3ST1</td>
<td>3.5</td>
<td>6.15E-13</td>
<td>1.93E-11</td>
<td></td>
</tr>
<tr>
<td>203645_s_at</td>
<td>CD183</td>
<td>3.5</td>
<td>5.54E-15</td>
<td>3.61E-13</td>
<td></td>
</tr>
<tr>
<td>202232_s_at</td>
<td>SF3X2</td>
<td>3.5</td>
<td>1.07E-12</td>
<td>3.10E-11</td>
<td></td>
</tr>
<tr>
<td>215072_at</td>
<td>SOD2</td>
<td>3.5</td>
<td>2.51E-13</td>
<td>8.83E-12</td>
<td></td>
</tr>
<tr>
<td>215049_s_at</td>
<td>CD183</td>
<td>3.4</td>
<td>1.50E-14</td>
<td>8.26E-13</td>
<td></td>
</tr>
<tr>
<td>228018_at</td>
<td>C20orf58</td>
<td>3.4</td>
<td>1.81E-15</td>
<td>1.34E-13</td>
<td></td>
</tr>
<tr>
<td>206697_s_at</td>
<td>HP</td>
<td>3.4</td>
<td>1.34E-09</td>
<td>1.51E-08</td>
<td></td>
</tr>
<tr>
<td>202357_s_at</td>
<td>BF</td>
<td>3.4</td>
<td>5.60E-14</td>
<td>2.56E-12</td>
<td></td>
</tr>
<tr>
<td>244467_at</td>
<td>NA</td>
<td>3.3</td>
<td>2.02E-08</td>
<td>1.85E-07</td>
<td></td>
</tr>
<tr>
<td>226804_at</td>
<td>FAM20A</td>
<td>3.3</td>
<td>7.45E-19</td>
<td>1.96E-16</td>
<td></td>
</tr>
<tr>
<td>206391_at</td>
<td>RARRES1</td>
<td>3.3</td>
<td>1.74E-14</td>
<td>9.37E-13</td>
<td></td>
</tr>
<tr>
<td>201042_at</td>
<td>TGM2</td>
<td>3.3</td>
<td>3.90E-21</td>
<td>3.19E-18</td>
<td></td>
</tr>
<tr>
<td>206504_at</td>
<td>CYP24A1</td>
<td>3.2</td>
<td>3.01E-08</td>
<td>2.33E-07</td>
<td></td>
</tr>
<tr>
<td>209969_s_at</td>
<td>STAT1</td>
<td>3.1</td>
<td>3.69E-12</td>
<td>9.23E-11</td>
<td></td>
</tr>
<tr>
<td>221477_s_at</td>
<td>SOD2</td>
<td>3.1</td>
<td>4.31E-23</td>
<td>6.02E-20</td>
<td></td>
</tr>
<tr>
<td>205729_at</td>
<td>OSMR</td>
<td>3.1</td>
<td>1.27E-14</td>
<td>7.30E-13</td>
<td></td>
</tr>
<tr>
<td>243206_at</td>
<td>P8C1</td>
<td>3.1</td>
<td>7.31E-10</td>
<td>8.93E-09</td>
<td></td>
</tr>
<tr>
<td>226261_at</td>
<td>FGG</td>
<td>3.0</td>
<td>1.54E-18</td>
<td>3.43E-16</td>
<td></td>
</tr>
<tr>
<td>209588_at</td>
<td>PNMA2</td>
<td>3.0</td>
<td>7.23E-10</td>
<td>8.85E-09</td>
<td></td>
</tr>
</tbody>
</table>

Mueller T et al, AJT 2008
The majority of the highest transcripts in deceased donor biopsies are associated with the acute phase response.

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>acute phase</th>
<th>ldiff DD vs LD</th>
<th>p-value</th>
<th>adj. p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERPINA3</td>
<td>26.1</td>
<td>1.00E-26</td>
<td>7.39E-23</td>
<td></td>
</tr>
<tr>
<td>FGB</td>
<td>16.2</td>
<td>3.34E-13</td>
<td>1.12E-11</td>
<td></td>
</tr>
<tr>
<td>FGG</td>
<td>16.9</td>
<td>1.39E-13</td>
<td>5.26E-12</td>
<td></td>
</tr>
<tr>
<td>RARRES1</td>
<td>11.3</td>
<td>1.08E-13</td>
<td>4.26E-12</td>
<td></td>
</tr>
<tr>
<td>TF</td>
<td>9.3</td>
<td>2.05E-16</td>
<td>2.01E-14</td>
<td></td>
</tr>
<tr>
<td>REG1A</td>
<td>7.2</td>
<td>5.32E-12</td>
<td>1.28E-10</td>
<td></td>
</tr>
<tr>
<td>SOD2</td>
<td>6.8</td>
<td>1.60E-17</td>
<td>2.27E-15</td>
<td></td>
</tr>
<tr>
<td>C1orf34</td>
<td>6.6</td>
<td>2.03E-13</td>
<td>7.29E-12</td>
<td></td>
</tr>
<tr>
<td>NNMT</td>
<td>6.0</td>
<td>4.64E-22</td>
<td>4.88E-19</td>
<td></td>
</tr>
<tr>
<td>LBP</td>
<td>6.0</td>
<td>3.62E-18</td>
<td>9.86E-17</td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>6.0</td>
<td>1.25E-10</td>
<td>1.93E-09</td>
<td></td>
</tr>
<tr>
<td>REG1A</td>
<td>5.8</td>
<td>4.33E-11</td>
<td>7.74E-10</td>
<td></td>
</tr>
<tr>
<td>SOD2</td>
<td>5.6</td>
<td>2.57E-20</td>
<td>1.26E-17</td>
<td></td>
</tr>
<tr>
<td>C1orf34</td>
<td>5.5</td>
<td>2.05E-25</td>
<td>7.54E-22</td>
<td></td>
</tr>
<tr>
<td>NNMT</td>
<td>5.5</td>
<td>6.32E-18</td>
<td>9.90E-16</td>
<td></td>
</tr>
<tr>
<td>LBP</td>
<td>5.4</td>
<td>9.74E-12</td>
<td>2.10E-10</td>
<td></td>
</tr>
<tr>
<td>CXCL2</td>
<td>4.7</td>
<td>7.17E-14</td>
<td>3.07E-12</td>
<td></td>
</tr>
<tr>
<td>STEAP4</td>
<td>4.3</td>
<td>3.40E-18</td>
<td>6.59E-16</td>
<td></td>
</tr>
<tr>
<td>PLTP</td>
<td>4.06E-11</td>
<td>7.32E-10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Cytokines / Chemokines / Type I interferons**

**Complement system**

**Coagulation system**
OSMR expression is increased with organ inflammation and injury (studies published in the GEO registry)

The red bar reflects the measured level of abundance of an individual transcript across the samples. The blue square represents rank order and gives an indication of where the expression of that gene falls with respect to all other genes on that array.

Kidneys of 1 week old mice who will develop polycystic kidneys due to lacking aquaporin-11 (AQP11)

Time course in mouse hearts following ligation of the left descending coronary artery (LAD)

Bleomycin effect on lungs of mice strains resistant (C3H/HeJ) and susceptible (C57BL/6J) to fibrosis

Kidneys of mice strains resistant (C3H/HeJ) and susceptible (C57BL/6J) to fibrosis

Lung inflammation and injury after exposure to LPS and mechanical ventilation (MV)
Expression levels of OSMR and 64 similar genes in 10 different conditions
Transcripts with similarity to OSMR (205729_at) across 10 different clinical settings

GeneName  Similarity  Common  1553530_e  0.91  ITGB1
205729_at  1.00  OSMR  201939_at  0.91  PLK2
226621_at  0.95  FGG  220337_at  0.90  FLJ11752
205488_s_  0.95  HSSST1  213519_s_  0.90  LAMA2
202440_s_  0.94  PLSCR1  228937_at  0.90  FLJ38725
225807_at  0.93  CIRL  203900_at  0.90  HOX
218963_at  0.93  PLSCR1  201042_at  0.89  TGM2
202439_s_  0.92  FAS  227529_s_  0.89  AKAP12
228153_at  0.92  IRBCD2  222549_at  0.89  CLDN1
236488_s_  0.92  RARB1  1553575_s  0.89  SPEC2
206391_at  0.92  RARRES1  1553568_s  0.89  ITGB1
209118_s_  0.91  TUBA3  201761_at  0.89  MTHFD2
202651_at  0.91  FAM34A  204273_at  0.89  EDNRB
221935_s_  0.91  MGCG33132  214895_s_  0.89  ADAM10
203885_s_  0.91  VCAM1  222872_s_  0.89  FLJ22333
232544_at  0.81  CD362  202075_s_  0.89  PLTP
211981_at  0.81  COL4A1  220875_at  0.89  MGP
201576_at  0.81  PON2  200742_at  0.89  DOCK11
224959_at  0.81  SLC5A2  200762_s_  0.89  CLU
231989_at  0.81  DKFZp586K2222  191877_s_  0.89  ECT2
210830_s_  0.81  PON2  208331_s_  0.89  C10orf18
239519_s_  0.81  ASNS  221702_at  0.89  DNAJC10
205047_s_  0.81  PDGFRA  218313_s_  0.89  GALNT7
203131_at  0.81  CDH8  227568_at  0.89  HECTD2
205532_s_  0.81  CDH8  220117_at  0.89  TJP2
228377_at  0.81  KLHL14  204326_at  0.89  SPO11
211960_at  0.81  COL4A1  218403_at  0.89  AKAP12
221781_s_  0.81  DNAJC10  233065_s_  0.89  HCD
226522_at  0.81  DKFZp586K2222  200207_at  0.89  ND
204061_at  0.81  PRKX  222294_s_  0.89  RAB27A
208701_at  0.81  PRKX  204060_s_  0.89  PRKX
211884_s_  0.81  FER1L3  201020_at  0.89  YWHAH

Class

Normalized Intensity Values
OSM signaling most likely via OSMR type II receptor in proximal tubule cells

Topics

1. Acute kidney injury and mortality

2. The transcriptome of the implant biopsy

3. Oncostatin M and its receptor in organ injury
WT Control kidney
Normal

WT Clamped kidney
Widespread tubular necrosis with complete loss of epithelium

Osmr-/- Control kidney
Normal

Osmr-/- Clamped kidney
Qualitatively similar tubular damage as in WT clamped, but not as severe
Clustering of kidneys from WT and Osmr-/- mice after ischemia-reperfusion or cecal ligation and puncture

n ≥ 3 samples per condition, triplicate measurements per gene, real-time PCR
Unsupervised cluster analysis of kidneys and hearts following renal unilateral ischemia-reperfusion

a) 24 Kidney samples
b) 8 Heart samples
Inflammation/injury

OSM

↑ OSMR ↑

↑ OSMR ↑

↑ OSMR ↑

↑ OSMR ↑
Sepsis $\rightarrow$ AKI $\rightarrow$ BD

- Tissue Injury $\rightarrow$
- DAMP $\rightarrow$
- Receptors $\rightarrow$
- Pathways $\rightarrow$

Cytokines / Chemokines / Type I interferons

Complement system $\cap$ Serpins $\cap$ Coagulation system

OSM $\uparrow$ OSMR $\uparrow$
• pathogens +/- non-pathogens
• cellular leakage of endogenous molecules/alarmins

• danger associated molecular patterns
• e.g. HMGB1, S100, uric acid, HSPs, dsDNA, ...

• extra- and intra-cellular innate pattern recognition receptors
• e.g. TLRs, NLRs/inflammasome

• pro-inflammatory pathways
• NFκB, MAPK, IRF, ...

• OSM is an early pro-inflammatory IL-6 family cytokine
• rapidly induced, secretion from neutrophils, macrophages and T cells

• OSMR is expressed on epithelial and vascular smooth muscle cells
• locally and systemically induced by inflammation and injury

• OSM/OSMR ligation induces signalling through pro-inflammatory pathways
• context-dependent OSM/OSMR stimulate a variety of biological activities

• induction and release of ...
• up-regulation and binding to ...

• induction of biological effects via ...
• release of ...
• binding to ...
• activation of ...
• induction of ...
Histology scoring in kidney and heart tissues of WT and Osmr-/- mice
MPO levels in heart tissue
(n = 3 to 4 animals per condition, ELISA)

MPO levels in lung tissue
(n = 3 to 4 animals per condition, ELISA)

Changes between sham and exp within the WT or within the Osmr-/- mice both for heart or lung were NOT significant
OSM induces OSMR in epithelial, smooth muscle and endothelial cells

OSMR is not induced by the cytokines IL6, LIF or hyper-IL6

(PTEC=proximal tubular epithelial, VSMC=vascular smooth muscle, HUVEC=human umbilical vein endothelial cells)

- PTEC: *p < 0.05 OSM vs. baseline; †p < 0.05 OSM vs. other cytokines or OSM + OSMR blocking antibody (gp130 Ab). NS – non significant.
- VSMC: *p ≤ 0.05 OSM vs. baseline; †p ≤ 0.05 OSM vs. other cytokine or OSM + gp130 Ab.
- HUVEC: †p < 0.05 OSM vs. baseline; †p ≤ 0.05 OSM vs. other cytokines or OSM + gp130 Ab. ‡p < 0.05 for serum starved (SS) vs. normal medium for SERPINs.
OSM signal transduction

IL6 signal transduction

OSMR levels in 22 different tissues from 10 to 12 week old C57/BL6 mice

OSMR levels in 12 different tissues from healthy humans
OSMR is predominantly expressed in the renal microvasculature

OSMR (green) in vascular smooth muscle of cortical arterioles (A) and peri-glomerular (G) vessels

OSMR co-localizes (yellow) with alpha smooth muscle actin (red) in smooth muscle cells of peri-glomerular vessels

OSMR largely does not co-localize with PECAM (red, endothelium), but is highly expressed in smooth muscle compartment (green) of renal arterioles (A)

OSMR (green) is expressed in peri-tubular vessels in outer medulla, medullary rays (Tubule, T)

OSMR (green) does not colocalize with N-cadherin (red, proximal tubules) in cortex

OSMR (green) does not colocalize with E-cadherin (red, distal tubules) in outer medulla
IL-6 Family Cytokine Receptors

See left, grey, Tanaka 2003: Thin horizontal lines and broad bars represent the conserved cysteine residues and WS motifs, resp. Thin arrows represent low-affinity binding of OSM to each receptor component, while thick arrows show high-affinity binding. Zigzag arrows indicate the relay of intracellular signalling cascades. X shows no signalling. In humans there are two types of OSMR complexes. While the gp130/OSMRβ is exclusively used by OSM, the gp130/LIFR complex is shared by OSM and LIF. In mice Osm uses only one receptor complex: gp130/OsMr β but not gp130/Lifr. Furthermore hOSM cannot bind the murine OsMr complex but instead binds to the murine gp130/Lifr. Type I OSM receptor is identical to the high-affinity LIFR; thus many overlapping biologic responses b/w hOSM and hLIF are mediated by the shared type I receptor (i.e. the LIF receptor), whereas OSM manifests its specific responses through the type II receptor. Curiously mOsm transduces its signals through the Osm-specific receptor consisting of gp130 and OsMr but does not use the type I Osm receptor (i.e. the Lifr).

See upper half, Arita 2008 on form of amyloidosis: The IL-6R comprises two gp130 and one IL-6R molecules. There are two types of OSMR Rs: type I is composed of gp130 and LIFR, whereas type II comprises gp130 and OSMRβ. The IL-31 receptor is composed of OSMRβ and IL31RA (left).

Structural model of OSMRβ depicting the functional domains and the sites of the missense mutations in FPLCA. FNIII, fibronectin type III-like domain; CBD, cytokine binding domain; Ig-like domain, immunoglobulin-like domain. AA numbers of the 979 amino acid OSMRβ protein are shown on the left of the figure. The missense mutations p.I691T and p.G618A are located within the extracellular FNIII domains (middle). OSMR Type II and IL-31 R signalling in normal and FPLCA keratinocytes. After binding of OSM or IL-31 to these receptors, the FNIII domains of both receptor subunits interact and signal transduction occurs. The missense mutations seen in our cases ofr FPLCA occur within the FNIII domains (black crosses), regions critical for receptor dimerization and subsequent signaling. Failure to form conformationally functional receptor complexes leads to reduced receptor signaling after stimulation with either OSM or IL-31.
1. **OSM** binds to gp130/OSMR complex, ligand binding leads to hetero-dimerization of the complex which causes sequestering of JAK1/JAK2/TYK2 to the receptor complex. Receptor-associated JAK molecules are phosphorylated and in turn JAKs/TYK phosphorylate the cytoplasmic domain of OSMR and gp130 which induces the recruitment of the GRB/SOS adaptor complex along with pre-existing STAT3. Both the MAPK and STAT pathway are activated leading to downstream activation of effector genes and post-translational modification to already pre-existing protein. 2. STAT3 binds to IL-6RE’s in front of the C/EBP promoter and acute phase gene promoters. 3. IL-1 and TNF signaling cause the movement of NF-kappaB into the nucleus. 4. Cytosolic glucocorticoid receptor binds to glucocorticoid and translocates into the nucleus. 5. MAPK/MEKK pathway induces phosphorylation of C/EBP beta. 6. Together, STAT3, NFkappaB, CEBP beta, and glucocorticoid initially activate the expression of IL6/OSM responsive genes. 7. Additionally, C/EBP delta has a STAT3 binding site and is subsequently activated by OSM/JAK/STAT signal transduction. 8. Late activation of C/EBP delta is required for maintaining activation of the acute phase genes.