Epigenetics and CKD

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I have the following relationships to disclose.

Potential Financial Conflicts of Interest

(1) Employment: No
(2) Stock ownership or options: No
(3) Patent royalties/licensing fees: No
(4) Honoraria and advisory fees: Kyowa-Hakko-Kirin, Astellas, Chugai, GSK, JT, Tanabe-Mitsubishi
(5) Research funding: Kyowa-Hakko-Kirin, Daiichi-Sankyo, Alexion, Astellas, Takeda, JT
Epigenetic factors

- DNA methylation
- Histone modification
  - Acetylation
  - Methylation
  - Phosphorylation
  - Ubiquitination
  - Sumoylation
- Histone variant
- microRNA
- Long non-coding RNA
- Chromosome conformation
Epigenetics: the study of persistent changes in gene expression that does not involve mutations of the underlying DNA.
Metabolic memory
Epigenetic changes
Long-term benefits of intensive glucose control for preventing ESKD: ADVANCE-ON

<table>
<thead>
<tr>
<th></th>
<th>Intensive</th>
<th>Standard</th>
<th>Favors Intensive</th>
<th>Favors Standard</th>
<th>Hazard Ratio (95% CI) P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>In-trial period</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>ESKD</td>
<td>7</td>
<td>20</td>
<td></td>
<td></td>
<td>0.35 (0.15 - 0.83) 0.02</td>
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<tr>
<td>Renal Death</td>
<td>17</td>
<td>20</td>
<td></td>
<td></td>
<td>0.85 (0.45 - 1.62) 0.62</td>
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<tr>
<td>ESKD or Renal Death</td>
<td>23</td>
<td>36</td>
<td></td>
<td></td>
<td>0.64 (0.38 - 1.08) 0.09</td>
</tr>
<tr>
<td><strong>Post-trial period</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>ESKD</td>
<td>22</td>
<td>33</td>
<td></td>
<td></td>
<td>0.65 (0.38 - 1.11) 0.11</td>
</tr>
<tr>
<td>Renal Death</td>
<td>31</td>
<td>33</td>
<td></td>
<td></td>
<td>0.91 (0.56 - 1.49) 0.71</td>
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<tr>
<td>ESKD or Renal Death</td>
<td>51</td>
<td>60</td>
<td></td>
<td></td>
<td>0.83 (0.57 - 1.20) 0.31</td>
</tr>
<tr>
<td><strong>Overall trial period</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ESKD</td>
<td>29</td>
<td>53</td>
<td></td>
<td></td>
<td>0.54 (0.34 - 0.85) &lt; 0.01</td>
</tr>
<tr>
<td>Renal Death</td>
<td>48</td>
<td>53</td>
<td></td>
<td></td>
<td>0.89 (0.60 - 1.31) 0.56</td>
</tr>
<tr>
<td>ESKD or Renal Death</td>
<td>73</td>
<td>95</td>
<td></td>
<td></td>
<td>0.75 (0.56 - 1.02) 0.07</td>
</tr>
</tbody>
</table>
5th September

Seminar 2: Basic Science; Diabetes

Chairs: Josephine Forbes & Melinda Coughlan

Karin Jandeleit-Dahm

Masaomi Nangaku

Carol Pollock
Hypoxic memory
Epigenetic changes
The Nexus of Acute Kidney Injury, Chronic Kidney Disease, and World Kidney Day 2009

Mark D. Okusa,* Glenn M. Chertow,† and Didier Portilla,‡ for the Acute Kidney Injury Advisory Group of the American Society of Nephrology
VAP-1 in pericytes enhances neutrophil infiltration into the IR-injured kidney by generating H$_2$O$_2$.
Clinical AKI with Adaptive Repair

Marker Level vs. Duration

- Injury
- Repair

Functional Marker Threshold
- Damage Marker Threshold

Basile, Nangaku et al for the ADQI XIII Work Group. JASN 2016
Clinical AKI with Maladaptive Repair

Marker Level vs Duration

- Injury
- Repair
- Functional marker level higher than at start
- Fibrosis
- Functional Marker Threshold
- Damage Marker Threshold

Basile, Nangaku et al for the ADQI XIII Work Group. JASN 2016
Renal hypoxia in the pathophysiology of the AKI-to-CKD transition

Nangaku et al. Nephron 2017

- Capillary rarefaction
- Hypoxia
- Epigenetic changes: DNA methylation, histone modification (e.g., H3K9me2↓ by KDM3A↑, H3K27me3↑ by KDM6B↓), chromosome conformational change, IncRNA (e.g., DARS-ASI↑), miRNA
- Fibrosis
Hypoxia
Uremia

Progression of kidney disease to ESRD.

CKD
Tubulointerstitial Fibrosis
Glomerular Sclerosis

ESRD
Atrophic Kidney

Modified from Mimura, Tanaka, & Nangaku. Semin Nephrol 2013
Hypoxia as the final common pathway to ESKD

Peritubular ischemia contributes more to tubular damage than proteinuria in immune-mediated glomerulonephritis

Double immunostaining showed pimonidazole-positive tubules (brown) located close to Kim-1–positive tubules (pink).
Hirakawa, Tanaka, Nangaku. J Diabetes Investig 2017

**HIF**

- **Normoxia**
  - HIF-α

- **Hypoxia or PHD inhibition**
  - HIF-α
  - HIF-β

**Defense against hypoxia**

- Proteasomal degradation
- Activation of HIF-downstream genes

**Nrf2**

- **Unstressed condition**
  - Nrf2

- **Stressed condition or KEAP1 inhibition**
  - Nrf2

**Defense against oxidative stress**

- Proteasomal degradation
- Activation of Nrf2-downstream genes
2016 Albert Lasker Basic Medical Research Award

Oxygen sensing – an essential process for survival

William G. Kaelin, Jr.  Peter J. Ratcliffe  Gregg L. Semenza
Progression of kidney disease to ESRD.

Normal → Kidney Disease → Hypoxia → Uremia → CKD:
- Tubulointerstitial Fibrosis
- Glomerular Sclerosis

ESRD → Atrophic Kidney

Modified from Mimura, Tanaka, & Nangaku. Semin Nephrol 2013
D-serine accelerates tubular senescence

γ-H2AX staining

Control

D-serine

γ-H2AX positive cells

SA-βG staining

Control

D-serine

SA-βG positive cells

Chirality

Okada, Nangaku et al. Sci Rep in press
Indoxyl sulfate suppresses HIF activity

IS: indoxyl sulfate

Tanaka, Nangaku et al. FASEB J 2013
Suppression of HIF and its targets by indoxyl sulfate

Huang et al. Kidney Int 2016
Indoxyl sulfate inhibits binding of HIF to its co-factor, p300

**Graph:**
- **Y-axis:** Corrected RLU
- **X-axis:**
  - IS
  - VP16
  - p300CH1/VP16

Legend:
- **-** indicates absence
- **+** indicates presence

Significance:
- **(##)** indicates high significance

Source: Tanaka, Nangaku et al. FASEB J 2013
Indoxyl sulfate induces HIF-inhibiting CITED2 protein

Tanaka, Nangaku et al. FASEB J 2013
The efficient displacement of HIF-1α from its complex with CBP/p300 by CITED2 is kinetically driven and proceeds through a transient ternary intermediate

Berlow et al. Nature 2017
Progression of kidney disease to ESRD.

Modified from Mimura, Tanaka, & Nangaku. Semin Nephrol 2013
Measurement of oxygen tension in the kidney utilizing a novel phosphorescence probe
Phosphofluorescent probe to detect hypoxia

Trichrome-Masson staining

Contralateral  I/R injured

Phosphofluorescence

Contralateral  I/R injured

# : P < 0.05 by paired two-tailed t-test

Hirakawa, Nangaku et al. Sci Rep 2015
S1 segment has higher oxygen tension compared with S2 segment

Hirakawa & Nangaku. *manuscript in submission*
Epigenetics

DNA methylation
miRNA and IncRNA
Histone modification
Chromosomal conformational change
Maternal protein restriction diet induced less weaning weight and hypermethylation of ribosomal DNA in offspring.
Ischemia-reperfusion injury decreased the genome-wide methylation level and the CpG methylation level.
Aberrant Rasal1 promoter methylation contributes to sustained fibroblast activation and AKI-to-CKD progression

Rasal1: Ras-Gap–like protein-1

Tampe et al. Kidney Int 2017
Epigenetics

DNA methylation
miRNA and IncRNA
Histone modification
Chromosomal conformational change
A clear advantage of targeting lncRNA rather than epigenetic-related enzymes or other non-coding RNA such as miRNA is the direct sequence specificity of the target site.

<table>
<thead>
<tr>
<th></th>
<th>miRNA</th>
<th>Epigenetic enzymes</th>
<th>lncRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td>Ubiquitous</td>
<td>Ubiquitous</td>
<td>Cell/tissue specific</td>
</tr>
<tr>
<td>Expression</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Therapeutic targeting</td>
<td>Not specific to a target gene</td>
<td>Not specific to a target gene</td>
<td>Sequence specific</td>
</tr>
</tbody>
</table>
RNA-seq revealed *DARS-AS1* as a hypoxia-induced lncRNA

Mimura, Nangaku et al. Physiol Rep 2017
DARS-AS1 is induced by HIF-1

Mimura, Nangaku et al. Physiol Rep 2017
Knockdown of *DARS-AS1* aggravates apoptotic cell death

Mimura, Nangaku et al.  
Physiol Rep 2017
Epigenetics

DNA methylation
miRNA and IncRNA
Histone modification
Chromosomal conformational change
Maternal H3K27me3 controls DNA methylation-independent imprinting

Inoue et al. Nature 2017
Epigenetic modification of expression of glucose transporter 3 (GLUT3) by hypoxia

Mimura, Nangaku et al. Mol Cell Biol 2012
Histone demethylation by hypoxia

Upregulation of KDM3A

KDM3A = Jmjd1a demethylase of H3K9me2

Mimura, Nangaku et al. Mol Cell Biol 2012
Regulation of GLUT3 expression by hypoxia

Mimura, Nangaku et al. Mol Cell Biol 2012
Cross-enhancement of ANGPTL4 transcription by HIF1 and PPAR β/δ

Inoue, Nangaku et al. Genome Biol 2014
Inoue, Nangaku et al. Genome Biol 2014
OMX super-resolution microscopy showed changes of the distribution of H3K9Me3 marks in NRK-52e cells by TGF-β1

H3K9Me3: repressive histone mark
NUP62: nuclear envelope protein nucleoporin 62

Hewitson et al. Front Pharmacol 2017
curcumin improves nephrosclerosis via suppression of histone acetylation

acetylated H3K9

Normal salt | High salt | High salt + curcumin

Serum creatinine

ChIP using anti-acetyl-H3K9

Muta et al. NDT 2016
increased EZH2 in the human fibrotic kidney

EZH2 (Enhancer of zeste homolog 2): histone methyltransferase of H3K27me
EZH2 mediates kidney fibrosis by downregulating expression of Smad7 and PTEN

Zhou et al. JASN 2016
Dznep attenuates renal fibrosis in obstructed kidneys

Dznep: inhibitor of EZH2

Zhou et al. JASN 2016
Amelioration of AKI-to-CKD transition by Dznep

Mimura, Hirakawa, Nangaku. *manuscript in submission*
Suppression of H3K27me3 by Dznep

Nuclear staining of tubular cells showed decreased staining of H3K27me3.

Mimura, Hirakawa, Nangaku. *manuscript in submission*
RNA-seq of tubules isolated by laser capture microdissection

- **Group 1**: 478 genes
- **Group 2**: 103 genes
- **Group 3**: 226 genes

Control, Vehicle I/R injury, Dznep I/R injury
Dznep suppressed TIMP2 expression in the kidney

Mimura, Hirakawa, Nangaku. *manuscript in submission*
RNA-seq: in vitro

HK2: human kidney-2

RPTEC: renal proximal tubular epithelial cell (human primary culture cells)

Mimura, Hirakawa, Nangaku. manuscript in submission
Dznep suppresses expression of TIMP2 via microRNA

Identified by small RNA-seq
Mimura, Hirakawa, Nangaku. manuscript in submission
Tetsuhiro Tanaka
(Nephrology & Endocrinology)

Reiko Inagi
(CKD Pathophysiology)