μRNAs: New frontiers in kidney disease

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HOW CAN WE MODERNIZE TOXICOLOGY?

Normal | Increased risk | Damage | GFR | Kidney failure | Death

Translational Biomarkers Transform Kidney Safety Assessment

KIM
KIM’s Friend
New KIM
**HOW CAN WE MODERNIZE TOXICOLOGY?**

Translational Biomarkers Transform Kidney Safety Assessment

Kidney Injury Molecule

- AJP-Renal, 2006
- JASN, 2007
- Nat Biotech, 2010
- Kidney Intl, 2009
- JASN, 2013

Fibrinogen

- Blood, 2011
- Am J Path, 2012

A Panel of MicroRNAs

- Tox Sci, 2012
- Clin Chem, 2013

Paradigm shift in Predictive Kidney Toxicity Screening

**Exposure** to toxic compounds

- Normal Epithelium
- Necrosis
- Apoptosis

Genome, Proteome & Secretome

Cellular Signaling

Molecular Mechanisms

Systems Analysis

Computational Modeling

TOXICO-RESPONSE MODELS & SIGNATURES

**Normal** – **Increased risk** – **Damage** – **Kidney failure** – **Death**

GFR

Comparison with gold standard

- Sensitivity
- Specificity

Fibrinogen

- Kidney Injury Molecule

Translational Biomarkers Transform Kidney Safety Assessment
OUTLINE

- Who we are and what do we do?
- What’s special about μRNAs
- Human profiling results
- Mechanistic studies with μRNAs
HISTORY OF CIRCULATING NUCLEIC ACID DETECTION

Discovery of free circulating nucleic acids detected in humans

1948

Use of circulating DNA in diagnosing cancer and monitoring treatment

1977

miRNA discovered in C. elegans

1989

miRNA and cancer

1993

Circulating miRNA discovered

2000

Circulating miRNA as biomarkers of diseases

2002

Current

Elevated levels of circulating DNA detected in serum of patients with autoimmune disease

Circulating DNA of tumor origin

Discovery of miRNA in humans

2008

Use of circulating miRNA for prevention, diagnosis, prognosis, and therapeutic monitoring

Weiland M et al, RNA Biol, 2012
(1) Transcription of pri-miRNAs from genes in the nucleus by RNA polymerase II

(1) Processing of primary transcript into 70 nt long pre-miRNA

(1) Export into cytoplasm in a Ran-GTP dependent manner through Exportin 5

(1) Cleavage of pre-miRNA by RNase III enzyme Dicer, into a 22 nt double stranded RNA composed of the mature miRNA “guide” strand and the low abundance miRNA* “passenger” strand

(1) Incorporation of mature miRNA into RNA Induced Silencing Complex (RISC)

Saikumar, Ramachandran and Vaidya, 2013
6. Packaging into multivesicular bodies (MVBs) that fuse with the plasma membrane and release as exosomes in a ceramide dependent pathway positively regulated by neutral sphingomyelinase 2 (nSMase2)

6. Encapsulation into high-density lipoprotein (HDL) particles which is repressed by nSMase2

6. Binding to RNA binding proteins, namely AGO-2 and nucleophosmin 1 (NPM1)

6. Incorporation into apoptotic bodies
NON-INVASIVE MICRO-MARKERS

Exosomal miRNAs - mast cells from Bone Marrow

miRNAs in HDL - Plasma

miRNAs bound to protein - Plasma

miRNAs in apoptotic bodies – *in vitro* in HUVEC cells

Cerebrospinal fluid
Tears
Saliva
Sputum
Breast milk
Pleural fluid
Blood/Plasma/Serum
Peritoneal fluid
Urine
Feces

Amniotic fluid
Vaginal fluid
Seminal fluid

Saikumar, Ramachandran and Vaidya, 2013
WHAT MAKES MIRNAS GOOD BIOMARKERS?

- **Sensitive:** immediate release before mRNA transcription and protein translation.
- **Specific:** tissue and disease specific regulation.
- **Dynamic range:** ~ 50,000 copies of miRNA/cell.
- **Translational potential:** highly conserved with a high degree of inter- and intra-species homology.
- **Stability:** more resistant to degradation than mRNA and proteins; and able to be detected in formalin fixed paraffin embedded tissues (although DNA is more stable, it is less tissue specific).
- **Accessible** in plethora of biological fluids allowing noninvasive detection.
- **Quantitative** by PCR amplification.

STABILITY

Plasma miRNAs:
- Harsh boiling conditions (>80°C)
- Low or high pH
- > 5 freeze thaw cycles
- Storage at room temperature for > 1 week

Urinary miRNAs:
- No degradation for up to 1 day at room temperature
- > 4 freeze thaw cycles
WHAT MAKES MIRNAS GOOD BIOMARKERS?

SPECIES CONSERVATION
μRNAs as mechanistic biomarkers of kidney disease

- Exosomal and circulating miRNAs have been shown to be mechanistic biomarkers in various cancers, organ damage and disease states.
- MiR-210: plasma and urinary biomarker for acute and chronic forms of injury and acute allograft rejection as well as ESRD. Modulates cellular response and mitochondrial metabolism in hypoxic conditions.
- MiR-155: a key regulator of the immune response and its expression is decreased in the urine and plasma in both acute and chronic kidney damage as well as in ESRD.
- MiR-21: silences metabolic pathways; its expression significantly increases in kidney and urine following damage.

Saikumar, Ramachandran and Vaidya, 2013
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HEALTHY

AKI

Pooled URINES

Pooled URINES

RNA ISOLATION

REVERSE TRANSCRIPTION

PRE-AMPLIFICATION

MIRNA PROFILING (1809 miRNAs)

<table>
<thead>
<tr>
<th></th>
<th>HEALTHY</th>
<th>AKI</th>
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<tbody>
<tr>
<td>Expressed [E]</td>
<td>345</td>
<td>281</td>
</tr>
<tr>
<td>Borderline-expressed [BE]</td>
<td>275</td>
<td>223</td>
</tr>
<tr>
<td>Non-expressed [NE]</td>
<td>1287</td>
<td>1402</td>
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</tbody>
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E = 19-30 (Ct); BE = 30-32 (Ct); NE = >32 (Ct)

Ramachandran et al., Clin Chem, 2013
miR-4640-5p
miR-21-5p
miR-4698
miR-4650-3p
miR-200b-3p
let-7d-5p
miR-23a-3p
miR-3679-5p
miR-4724-5p
miR-4301
let-7b-5p
let-7c
miR-191-5p
miR-373-5p
miR-1301
miR-320b
miR-3620-3p
Expression levels of miR-21, 200c, 423 and 4640 are significantly different in patients AKI (n=117) as compared to healthy volunteers (n=97).
The combined cross-validated area under the receiver operator curve for miR-21, -200c, -423 and -4640 was computed to be 0.91.
WHICH NORMALIZER TO USE?
WHAT IS THE TEMPORAL VARIABILITY OF μRNAs

Ramachandran et al., Clin Chem, 2013
DRUG-INDUCED KIDNEY TOXICITY

- All 70 patients had high ALT (>150 IU/L) and received NAC treatment.
- Expression levels of miR-21, 200c and 423 are significantly higher in patients with Acetaminophen-induced kidney toxicity.

Control vs. SCr > 1

<table>
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<tr>
<th></th>
<th>miR-21</th>
<th>miR-200c</th>
<th>miR-423</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.87</td>
<td>0.82</td>
<td>0.83</td>
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</table>

Collaboration with Dr. Dan Antoine, Center for Drug Safety Science, UK
LONGITUDINAL STUDY

Urinary levels of miRNA-21 and miRNA-423 are significantly higher in patients admitted to the ICU who develop AKI which progresses to Renal Replacement Therapy (RRT) or death as compared to those who do develop AKI but do not require RRT as well as those who do not develop AKI.

Collaboration with Dr. Jay Koyner, Univ of Chicago
Can we perform small RNA sequencing in the urine?

Healthy Prostate Cancer

miR-451
miR-31
miR-185
miR-874

Collaboration with Dr. Martin Sanda, Emory Univ
OUTLINE

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MIR-155 KO ARE SUSCEPTIBLE TO KIDNEY TOXICITY

**Serum Creatinine (mg/dL)**
- C57BL/6J
- miR-155-/-

**Acute Tubular Injury Score**
- +/+ vs -/-

**Kim-1 (fold change)**
- +/+ vs -/-

**TUNEL+ nuclei/field of view**
- +/+ vs -/-

**Hours post Cisplatin injection**
- 0, 24, 48, 72, 96, 120
• We conducted a screen of the entire miRNome in human urine and have identified miR-21, miR-200c, miR-423, and miR-4640 as sensitive (and noninvasive) biomarkers of kidney damage.

• miR-155 plays a protective role in cisplatin-induced kidney toxicity.

• Extracellular RNAs have an immense potential as biomarkers.

• Understanding the biology of extracellular RNAs is likely to yield in newer insights in kidney health and disease.

• Will there be a unique and distinct panel of miRNA for subtype of kidney damage?

• How will a panel of miRNAs and proteins perform as biomarkers for early detection?

• Can we reduce kidney damage by targeting candidate miRNAs?