µRNAs: New frontiers in kidney disease

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OUTLINE

Who we are and what do we do?
- What’s special about µRNAs
- Human profiling results
- Mechanistic studies with µRNAs

HOW CAN WE MODERNIZE TOXICOLOGY?

Translational Biomarkers Transform Kidney Safety Assessment

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

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

Translational Biomarkers Transform Kidney Safety Assessment

Kidney Injury Molecule

Fibrinogen

A Panel of MicroRNAs

Paradigm shift in Predictive Kidney Toxicity Screening

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HISTORY OF CIRCULATING NUCLEIC ACID DETECTION

Discovery of free circulating nucleic acids in humans

Use of circulating DNA in diagnosing and monitoring treatment

\( \epsilon \)RNA discovered in C. elegans

\( \mu \)RNA and cancer

Circulating \( \epsilon \)RNA discovered

Circulating \( \mu \)RNA as biomarkers of disease

Elevated levels of circulating DNA detected in sera of patients with atherosclerosis

Circulating DNA of tumor origin

Discovery of \( \epsilon \)RNA in humans

Use of circulating \( \epsilon \)RNA for prevention, diagnosis, prognosis, and therapeutic monitoring
(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

Saikumar, Ramachandran and Vaidya, 2013

Non-Invasive Micro-MARKERS

Exosomal miRNAs - most cells form Bone Marrow
miRNAs in HDL - Plasma
miRNAs bound to protein - Plasma
miRNAs in apoptotic bodies - in vitro in HCSC cells

Cerebrospinal fluid
 Tears
 Salivary
 Squamous

Breast milk
Mammary fluid
Blood/Plasma/Serum
Pancreatic fluid

Urine
Sweat

Amniotic fluid
Vaginal fluid
Seminal fluid

Sources: Ramachandran and Vaidya, 2013
WHAT MAKES MIRNAS GOOD BIOMARKERS?

- Sensitive: immediate release before miRNA 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 formal fixed paraffin embedded tissues (although DNA is more stable but 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

- miR-21: plasma and urinary biomarker for acute and chronic forms of injury and acute allograft rejection as well as ESRD.
- miR-155: a key regulator of the immune response and its expression is decreased in the in 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.

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 in 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.
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HEALTHY AKI

RNA ISOLATION

E in Healthy BE in Healthy NE in Healthy

248

58

39

28

E in both

378 miRNAs selected

378 miRNA Profiling

8 excluded

54 excluded

8 excluded

miR-502-5p

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

Ramachandran et al., Clin Chem, 2013
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 \( \mu \)RNAs

![Graphs showing temporal variability of \( \mu \)RNAs](image)

Ramachandran et al., Clin Chem, 2013

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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.

<table>
<thead>
<tr>
<th></th>
<th>Control vs. SCr &gt; 1</th>
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<tbody>
<tr>
<td>miR-21</td>
<td>0.87</td>
</tr>
<tr>
<td>miR-200c</td>
<td>0.82</td>
</tr>
<tr>
<td>miR-423</td>
<td>0.83</td>
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</tbody>
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Collaboration with Dr. Dan Antoine, Center for Drug Safety Science, UK

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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 not 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.

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MIR-155 KO ARE SUSCEPTIBLE TO KIDNEY TOXICITY

Hours post Cisplatin injection
• 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

SUMMARY

CONCLUSION

QUESTIONS

• 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?

Biomarkers
Systems Tox
Mechanisms
µRNAs

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