Novel Diagnostic and Prognostic Biomarkers for Prostate Cancer

Exiqon Diagnostics
Anne Karin Rasmussen, Ph.D.
Prostate cancer (PCa) is the most common cancer in men

- 1 million new cases per year
- 300,000 deaths per year
- Prostate cancer is curable if detected in time

- Prostate cancer risk increases with age
- Almost all men develop prostate cancer during their lifetime

Prostate cancer affects men over 50

- Age 50-59
- Age 60-69
- Age 70-79

Autopsy study
Not all men with prostate cancer need treatment

2 types of prostate cancer

Harmless
No effect on quality of life or lifespan
No treatment needed

Aggressive
Deadly: spreads fast to other parts of the body
Needs immediate treatment
Early detection is crucial

Early detection is crucial
Today: Diagnosis and treatment selection rely on PSA test and biopsy

**When cancer is suspected**
- Blood test measures the **PSA** level (Prostate Specific Antigen)

**When PSA level is elevated (PSA ≥3 ng/mL)**
- TransRectal UltraSound (TRUS) guided biopsy - small sample of prostate tissue

**Look at cancer cells under the microscope**
- **Harmless**
  - No treatment
- **Aggressive**
  - Treatment
Today: Too many unnecessary biopsies on healthy men

PSA blood test is not reliable

- 7 in 10 men with raised PSA are CANCER FREE
- 1 in 10 men with normal PSA DO have cancer

PSA grey-zone (3-10 ng/mL)
- Very low predictive value (25%)
- About 40% of performed tests falls into this category

Biopsy carries a risk
- Invasive procedure
- Risk of infection/bleeding/sepsis

Better non-invasive tests for prostate cancer are needed
MicroRNAs make excellent biomarkers

- Regulate gene expression (mRNA transcription)
- Important regulatory roles in many diseases
- Actively secreted from cells into the circulation – act as “signalling” molecules
- Highly stable in clinical sample preparations

Adapted from Guire et al. Clinical Biochemistry 2013
Exosomes stabilize microRNAs in a range of biofluids

- Carriers for proteins, DNA and RNA (microRNA, piRNA, IncRNA & mRNA)
- MicroRNAs are selectively sorted into exosomes
- Tumor-derived exosomes - provides disease “fingerprints” (RNA levels)
- Promising non-invasive biomarkers for early detection of cancer
Analyzing microRNAs in urine is challenging

- **Urine**
  - Few microRNAs present in cell-free urine - *even lower amounts than in serum / plasma*
  - Contains inhibitors of RT-PCR

- **MicroRNA**
  - Short length
  - Diverse GC content
  - Closely related family members
Exosomes are of key importance to detection of microRNAs in urine

- miRCURY™ Exosome Isolation Kit enables detection of more microRNAs in urine

![Bar chart showing microRNAs detected on miRNome panel I for different urine starting volumes.]
miRCURY™ Exosome Isolation Kit increases microRNA signals

- Easy short protocol
- Concentrate or increase starting volume of biofluid
- Without co-purification of inhibitors
Challenging samples require a sensitive and specific detection method

- LNA™ Universal RT microRNA PCR System enables robust biofluid microRNA profiling

**Step 1: First-strand synthesis (RT)**

- Mature microRNA
- Polyadenylation
- Reverse transcription

**Step 2: Real-time PCR amplification**

- miR-specific forward primer
- Two LNA™ enhanced microRNA specific primers
- SYBR Green detection

3’ degenerate anchor

5’ universal tag

miR-specific reverse primer
Exiqon’s qPCR offers high sensitivity and linearity - ideal for microRNA analysis in biofluids

![Graph showing Cq values for different Total RNA inputs in RT reaction for various microRNAs such as hsa-let-7d-5p, hsa-miR-145-5p, hsa-miR-194-4p, hsa-miR-1, hsa-miR-133a, and hsa-miR-451a.]
Exiqon’s qPCR system offers perfect specificity within microRNA families

Adapted from Mestdagh et al.
Nature Methods 2014
Highly sensitive microRNA detection in urine

<table>
<thead>
<tr>
<th>Exosome Isolation</th>
<th>RNA Isolation</th>
<th>Real-time PCR</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRCURY™ Exosome Isolation Kit</td>
<td>miRCURY™ RNA Isolation Kit - Cell &amp; Plant</td>
<td>miRCURY LNA™ Universal RT microRNA PCR Assay</td>
<td>Simple quantification without the need for normalization</td>
</tr>
<tr>
<td>3 ml cell-free urine</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt; 2 hours</td>
<td>20 min</td>
<td>3 hours</td>
<td>10 min</td>
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Prostate cancer diagnosis
# Urine microRNA biomarkers for Prostate cancer

## - Overview of the Study Design

<table>
<thead>
<tr>
<th>DISCOVERY PHASE</th>
<th>VALIDATION PHASE</th>
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</thead>
<tbody>
<tr>
<td><strong>Genome wide screening</strong></td>
<td><strong>Validation set miRNA signature</strong></td>
<td><strong>External validation set miRNA signature</strong></td>
</tr>
<tr>
<td>Discovery screening on subset</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Limited sample size:</strong> 50 individuals</td>
<td><strong>Larger sample size:</strong> 222 individuals (22 BPH, 200 PCa)</td>
<td><strong>Large sample size:</strong> 227 individuals (22 BPH, 205 PCa)</td>
</tr>
<tr>
<td>miRNome PCR panels 752 microRNAs</td>
<td>Custom Pick-&amp;-Mix PCR panels (92 microRNAs)</td>
<td>Custom PCR panels microRNA signature</td>
</tr>
<tr>
<td>Identify subset of relevant microRNAs</td>
<td>Identify candidate microRNA biomarkers &amp; endogenous controls</td>
<td>Signature identification</td>
</tr>
</tbody>
</table>

**BPH** = Benign Prostatic Hyperplasia  
**PCa** = Prostate Cancer
A novel 3-microRNA diagnostic signature

**DISCOVERY PHASE**

Cohort 1

**VALIDATION PHASE**

Cohort 2

AUC = 0.955
P < 0.001

AUC = 0.892
P < 0.001
The 3-microRNA signature identifies individuals with prostate cancer in the PSA grey-zone group

Cohort 2 sub-population (PSA< 10 ng/mL)

Today: PSA grey-zone (3-10 ng/mL)

- 75% false positive
- About 40% of performed tests falls into this category

Sensitivity: 0.88
Specificity: 0.95
Cut-off: >6

AUC = 0.907
P < 0.001
Exiqon’s urine test for reliable detection of prostate cancer

Superior accuracy of Exiqon test will reduce number of false positives

<table>
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<th>Improved cancer detection based on urine analysis</th>
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<td>PSA test</td>
</tr>
<tr>
<td>7 in 10 men with raised PSA are cancer free</td>
</tr>
<tr>
<td>Exiqon’s objective</td>
</tr>
<tr>
<td>Only 1 in 10 men with a positive test result is cancer free</td>
</tr>
</tbody>
</table>

Vision

- To bring a simple and precise test to the clinic
- A supplement to the PSA test
- Targets about 40% of patients with ambiguous PSA results

Impact

- Increased patient safety
- Fewer invasive biopsies
- Considerable savings for the healthcare system
Today: Treatment selection rely on biopsy

When cancer is suspected

Blood test measures the **PSA** level (Prostate Specific Antigen)

When PSA level is elevated (PSA ≥3 ng/mL)

TransRectal UltraSound (TRUS) guided biopsy - small sample of prostate tissue

Look at cancer cells under the microscope

- Harmless
  - No treatment
- Aggressive
  - Treatment
Today: Biopsy results are frequently inconclusive or wrong

**Biopsy results are unreliable**
- Results are subjective
- Results are frequently wrong – not reflecting the true cancer state

**Consequences**
- Unnecessary overtreatment
- Misdiagnosis - potentially fatal delay in treatment
  - reduced quality of life (impotence/incontinence)

**Better tests are needed to guide treatment decisions**
Study design
- to find prognostic biomarkers

- Inclusion criteria
  - Patients with previous radical prostatectomy with 5-10 year follow up

- Endpoint
  - Biochemical (PSA) recurrence as a measure of tumor aggressiveness

- Clinical questions
  - Signature that stratifies patients into high and low risk groups with significant different outcomes?
microRNA profiling of FFPE tissue samples
- Cohorts 1 and 2

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<th>RNA isolation</th>
<th>Real-time PCR</th>
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<td>“Simulated biopsies” 1.5 mm punch from FFPE block</td>
<td>miRCURY LNA™ Universal RT microRNA PCR</td>
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Formalin-Fixed, Paraffin-Embedded tissue (FFPE)
# Tissue biomarker discovery and validation - overview

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<td><strong>Genome wide screening</strong></td>
<td><strong>Validation</strong></td>
<td><strong>Validation</strong></td>
</tr>
<tr>
<td><strong>RP cohort 1:</strong> 126 RP samples</td>
<td>31 relapse, 95 non-relapse</td>
<td>31 relapse, 95 non-relapse</td>
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<td>Identify candidate microRNA biomarkers + endogenous controls</td>
<td>Validate candidate microRNA biomarkers</td>
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</tr>
<tr>
<td>miRCURY LNA™ microRNA PCR Panels I + II 752 microRNAs</td>
<td>miRCURY LNA™ microRNA Custom PCR Panels 94 microRNAs</td>
<td>GSE21036: Agilent Human microRNA Microarray 2.0 – 368 microRNAs</td>
</tr>
</tbody>
</table>

*Taylor et al., Cancer Cell, 2010;18:11-22*
Results: A novel 3-microRNA prognostic classifier

- First demonstration of microRNA signatures with significant independent prognostic value in three independent cohorts

**DISCOVERY**
- RP Cohort 1

**VALIDATION**
- RP Cohort 2
- RP Cohort 3

**Note:** Biochemical Recurrence-Free Survival (RFS) after Radical Prostatectomy (RP)

- Formalin fixed tissue, miRCURY LNA™ PCR Assay
- Snap frozen tissue, array technology

Paper: Kristensen et al., Oncotarget, 2016
Urine test improves diagnosis

- Biomarker profile determines next step

Urine analyzed by qPCR test (microRNA biomarker)

- Fewer false positives and false negatives
- Fewer unnecessary invasive biopsies

Biopsy

Biopsy test supports treatment decisions

- Better discrimination between harmless and aggressive prostate cancer
- Avoid overtreatment

Analyze biopsy by qPCR test (microRNA biomarker)

Harmless

- No treatment

Aggressive

- Treatment

Tomorrow: Improved microRNA-based diagnostic and prognostic biomarkers
## Acknowledgements

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- Jacob Fredsøe

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**Paper: Novel diagnostic and prognostic classifiers for prostate cancer identified by genome-wide microRNA profiling**
- Kristensen et al., Oncotarget, 2016
Thank you for your attention!
Exiqon Diagnostics