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Poster # 1943

A microRNA signature in urinary exosomes for diagnosis of prostate cancer

Thorarinn Blondal[1], Anne I. Rasmussen[1], Anni R. Thomsen[1], Michael Borre[2], Jacob Fredsøe[3], Ditte Andreasen[1], James Catto[4], Torben Falck Ørntoft[3], Karina D. Sørensen[3] and Peter Mouritzen[1].

[1] Exiqon A/S, Vedbaek, Denmark, Depts of [2]Urology and [3]Molecular Medicine, Aarhus University Hospital, Denmark, [4]Department of Oncology, The Medical School, Sheffield, UK

Presented at the AACR conference, April 2016

Introduction

Improved diagnostic tests for prostate cancer are needed

- Current practice: antibody based detection of circulating prostate specific antigen (PSA) in blood
- High rate of false positives and negatives
- Diagnosis requires invasive FNA (Fine-Needle Aspiration) biopsies

→ A non-invasive test with improved specificity is urgently needed

MicroRNAs as non-invasive biomarkers

- MicroRNAs are stable in a range of biofluids
- Involved in many diseases, including roles as oncogenes and tumor suppressors in cancer
- Used as diagnosis, prognosis, treatment response and safety biomarkers

→ microRNAs are excellent non-invasive biomarkers for a range of diseases

Exosomes stabilize and transfer microRNAs between cells

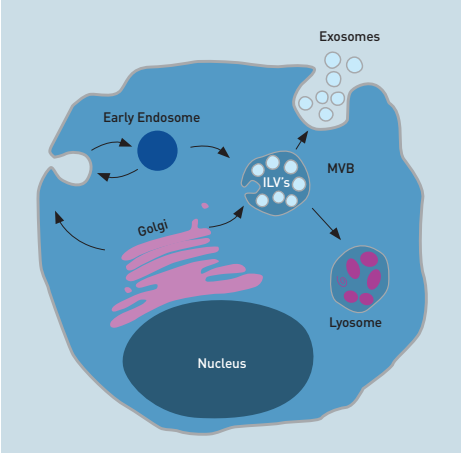


Figure 1.

- Exosomes are nanovesicles 40 - 140 nm in diameter
- Actively released by a wide range of cell types to the extracellular milieu under normal and pathological conditions
- Present in a wide range of biofluids
- Carry genetic information from the cell of origin, including microRNA

Urinary exosomes: new liquid biopsies for cancer

- Exosomes from neoplastic cells carry potentially arrays of oncogenic molecules including proteins and microRNAs
- The unique exosomal microRNA signature may reveal the cell of origin and the condition of those cells

→ Promising non-invasive microRNA biomarkers for early detection of malignancy

Challenges and Solutions for analysis of microRNAs in Biofluids

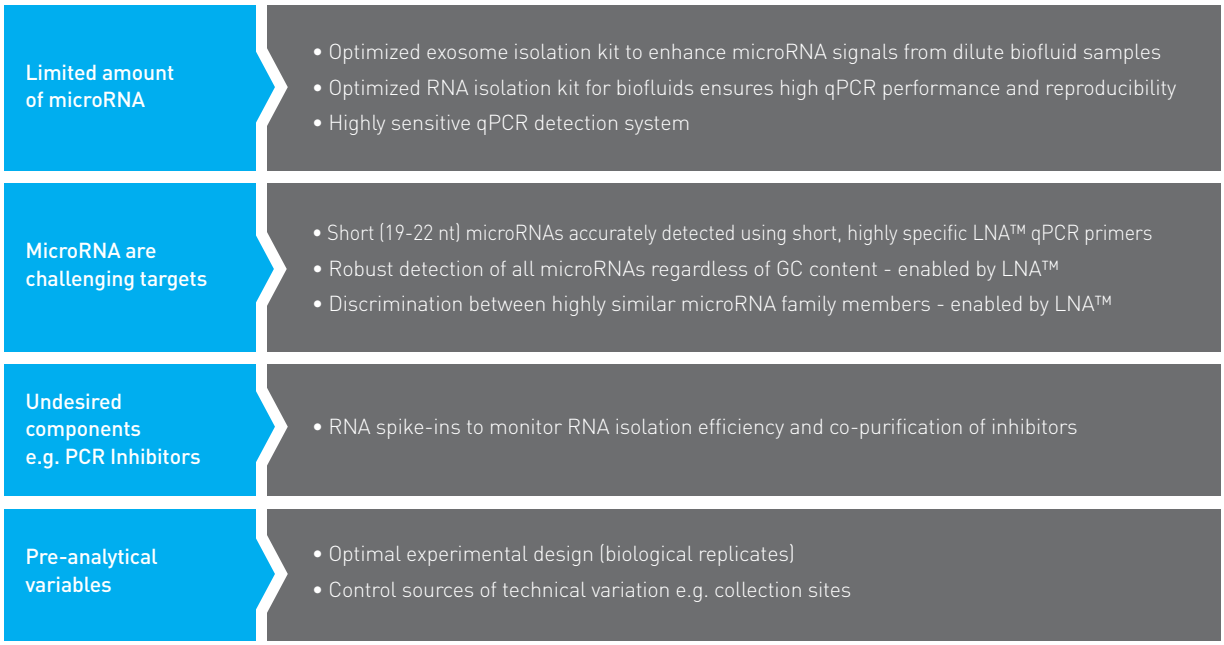


Figure 2.

Methods

Technologies to enable microRNA biomarker analysis in liquid biopsies

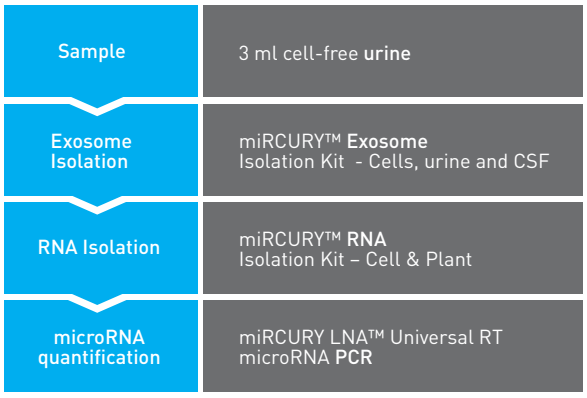


Figure 3.

Exiqon has developed technologies to fulfil these key requirements:

- Methods suitable for **clinical liquid biopsies** (serum, plasma, urine, CSF etc.) collected using standard protocols
- Exosome precipitation using a rapid method (< 1 hour), requiring only **low speed centrifugation**
- Optimized sample preparation to **minimize carryover of inhibitory compounds** in biofluids
- Procedures for **rigorous QC** of liquid biopsy samples
- Highly **sensitive** detection system to handle the very low level of RNA found in biofluids
- Highly **specific** detection method to discriminate between closely related microRNA family members
- Detection method optimized for detection of **short** microRNA sequences

miRCURY™ Exosome Isolation Kit

Exosome isolation enables detection of more microRNAs in urine

Exosome isolation enables a larger starting volume of biofluid to be used, increasing signals.

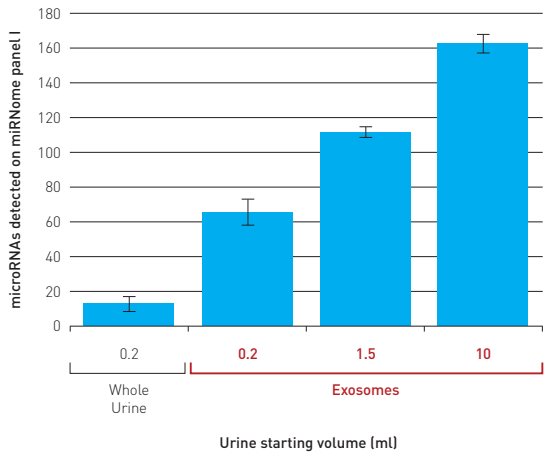


Figure 4.

Vesicles of the correct size are recovered in the exosome pellet

Nanosight measurements demonstrate that vesicles of a size range compatible with exosomes are enriched from urine in the pellet.

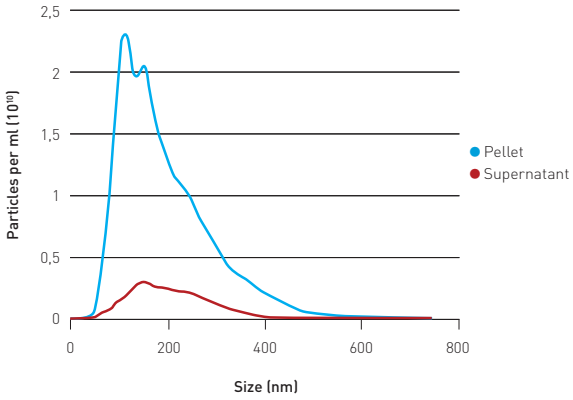


Figure 5.

Rigorous QC of liquid biopsies

qPCR-based QC procedures optimized for biofluids

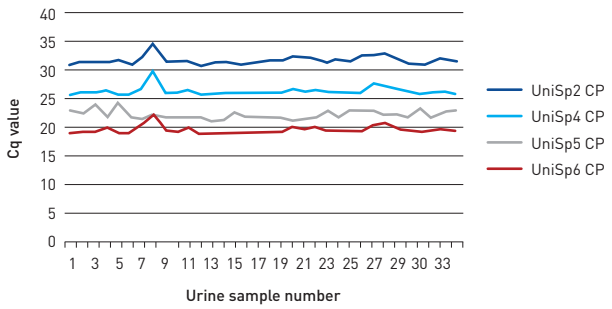
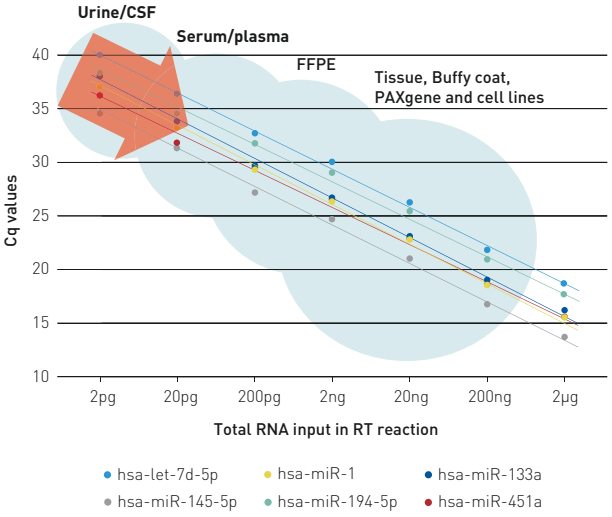


Figure 5.

A range of RNA spike-ins are detected by LNA™ qPCR assays to monitor RNA isolation efficiency, inhibitors, and detect outlier samples.

High sensitivity and linearity - Ideal for microRNA analysis in liquid biopsies

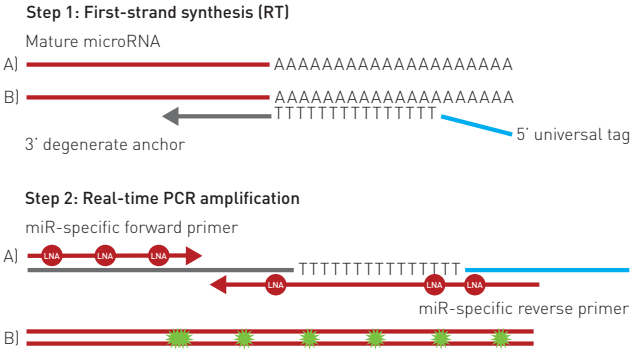


- Sensitive assays are crucial, due to the low RNA content of biofluids
- Exiqon's miRCURY LNA™ microRNA PCR assays are wet-lab validated to have sensitivity and linearity over a wide range of RNA inputs, including biofluids
- Red arrow indicates improvement of microRNA detection from dilute biofluid samples e.g. urine when using the miRCURY™ Exosome Isolation Kit

Figure 7.

A robust system for accurate microRNA analysis - Validated on biofluids

- In the largest cross-platform comparison study ever (miRQC), Exiqon's PCR system was the only microRNA analysis platform to combine both high sensitivity and specificity (Mestdagh et al., Nature Methods 11(8):809-15, 2014)
- We have used the miRCURY LNA™ Universal RT microRNA PCR System to analyze microRNAs in thousands of biofluid samples including serum, plasma and urine



Polyadenylation

Universal Reverse Transcription:
speed and convenience

LNA™ in two microRNA specific primers:
sensitivity and specificity

SYBR Green detection:
verification of amplicon

Figure 8.

Results

Study overview - microRNA biomarker discovery

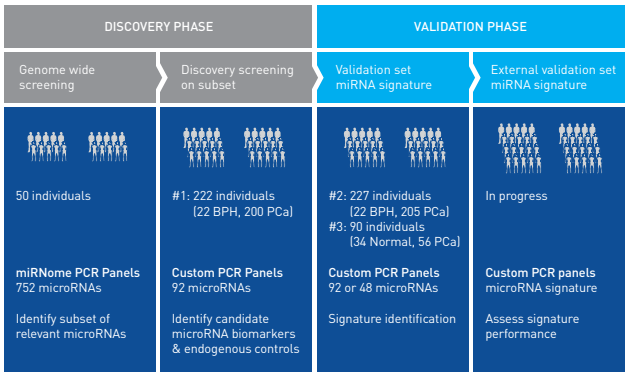


Figure 9.

Urine sample collection and analysis:

Urine samples collected by hospitals in Denmark

- 3 ml fresh urine (without stabilizer) was centrifuged to remove cell debris
- Storage in cryotubes at -20 °C (short term) then -80 °C (long term)
- Exosome and RNA isolation followed by microRNA qPCR analysis, using the methods shown in Figure 3

Urine samples collected by hospitals in the UK

- Prostate massaged fresh urine (without stabilizers) was centrifuged to remove cell debris
- RNA isolation without exosome isolation followed by microRNA qPCR analysis, using the method shown in Figure 3

Genome wide microRNA profiling of cell-free urine samples:

- Healthy individuals (Benign Prostatic Hyperplasia) and patients with Prostate Cancer (Stage I-IV)
- A subset of relevant microRNAs were selected for subsequent discovery screening

microRNA biomarker discovery and validation on cell-free urine samples:

- Discovery on 222 individuals
- Validation on 227 and 90 individuals from two independent retrospective cohorts
- Individuals with localized or advanced localized Prostate Cancer, as well as Healthy Controls (Benign Prostatic Hyperplasia)

Diagnostic microRNA signatures for prostate cancer in urine

- **Differentially regulated microRNAs** in urine from prostate cancer individuals were identified (Cohort 1, DK)
- **Signatures with diagnostic potential for prostate cancer** have been identified using different combinations of these microRNAs
- **Three-microRNA signature:** high Area Under the Curve (AUC) was validated in two independent cohorts – one using sample collection and isolation protocols identical to the discovery cohort (cohort 2, DK) and one prostate massaged cell free urine without exosome isolation (cohort 3, UK) (Figure 10a)
- The three microRNA signature shows high performance within the intended-use-population (cohort 2 sub-population, DK) (Figure 10b)

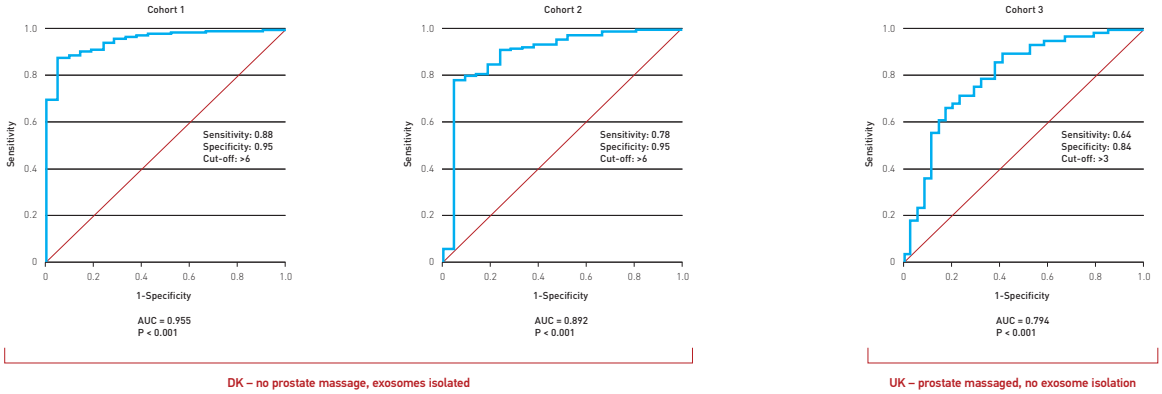


Figure 10a.

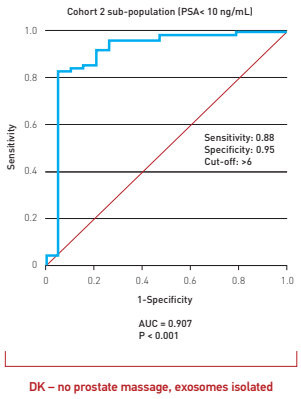


Figure 10b.

A new diagnostic test (non-invasive) for prostate cancer - intended use: men with intermediary PSA levels

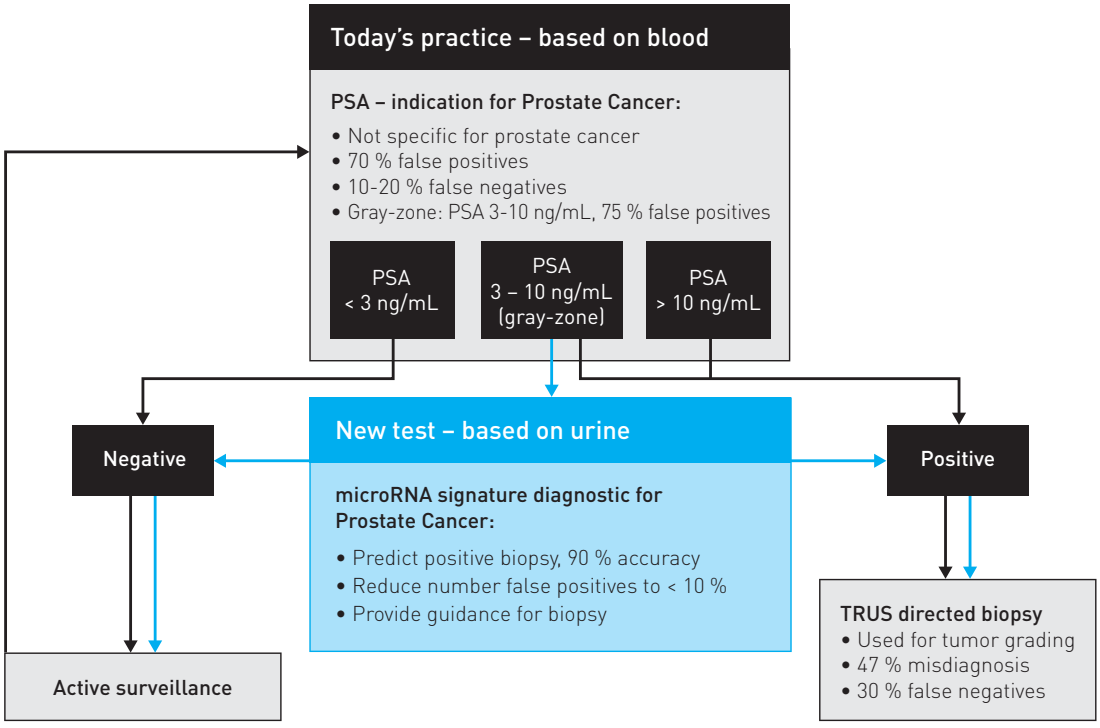


Figure 11.

Conclusions

- **Requirements** for microRNA analysis in biofluids:
  - RT-qPCR system with high sensitivity and specificity
  - Rigorous sample QC and standardization
  - Enrichment of exosomes is preferred in dilute biofluids
- **Exosome isolation** enables detection of more microRNAs in dilute biofluids
- The **methods** developed for sample preparation and LNA™-enhanced microRNA qPCR analysis have been successfully applied in **cell-free urine**
- microRNAs in cell free urine are promising **non-invasive biomarkers in prostate cancer diagnosis**
- **A three-microRNA signature** has been discovered
- The three-microRNA signature has been **validated in independent cohorts**

Acknowledgements: Nanosight data kindly provided by iNano (Dr. Ken Howard, Aarhus University).

Outside North America
Phone: +45 45 65 09 29
Fax: +45 45 66 18 88

North America
Phone: +1 781 376 4150
Fax: +1 781 376 4152
Toll free (US & Canada): +1 888 miRCURY

www.exiqon.com

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925112 - v1.0 - 04/2016