O13 A duplex quantitative real-time PCR assay for the detection of small non-coding RNA in urinary extracellular vesicles

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Introduction & Objectives: Extracellular vesicles (EVs) have emerged as a novel promising source of cancer bio-markers. EVs are produced and shed into different body fluids including blood and urine by virtually any normal or malignant cell. It is evident that the molecular content of EVs is highly resemblant of their cell of origin. Therefore, tumor cell originating urinary EVs can be explored as an rich source of minimally invasive, liquid biopsy RNA bio-markers for prostate cancer and other urological malignancies.
In this study, we build upon our previous work to develop a quantitative real-time PCR (qPCR) assay for the simultaneous detection of small non-coding RNA present in urinary EVs in order to evaluate their use as minimally invasive prostate cancer bio-markers.

Materials & Methods: We developed custom TaqMan Advanced assays (TermoFisher) against candidate biomarker microRNAs, tRNA fragments (tRFs), snoRNAs and snoRNA-derived RNAs (sdRNAs) and tested their performance by absolute quantification qPCR. Total RNA was isolated directly from urinary EVs captured with a precipitation kit (Norgen Biotek). cDNA was synthesized from the obtained total RNA or from equimolar mix of synthetic small RNA targets using TaqMan advanced universal cDNA synthesis kit (ThermoFishrer).

Results: To enable the simultaneous detection of two different RNA targets in the same PCR reaction, we designed TaqMan probes with either FAM or VIC labeling and tested their performance against a mix of synthetic RNA templates and in total RNA material isolated from urinary EVs. To establish efficiency and accuracy, all assays were tested as uniplex reaction with only FAM-labeled or only VIC-labeled probe in the reaction mix. Subsequently, duplex reactions including different combinations of FAM and VIC assays were performed and efficiency and accuracy were compared to the uniplex setting. After optimization, best performing duplex combinations were tested against total RNA derived from urinary EVs.

Conclusions: Our results demonstrate that duplex qPCR is an efficient method for the simultaneous detection of different small RNAs in the same reaction mix. This makes possible the evaluation of candidate small RNA biomarker combinations in clinical urine samples when the amount of starting material is limited and opens the door to the development of a small RNA-based, minimally invasive, liquid biopsy test for prostate cancer and other urological malignancies.